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BEARING ON HEREDITY AND VARIATION

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OENOTHERA CANTABRIGIANA

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University of Michigan, Ann Arbor, Michigan

Received March 18, 1940

OENOTHERA CANTABRIGIANA (figures 1 and 2) is a structural hybrid closely related to *Oenothera biennis* but with the interesting genetical peculiarity that the pollen and egg lethals are reversed in position from those of *biennis*. A rubens-like complex is carried by the egg and an albicans-like complex is carried by the pollen. It is as though there had been a crossover of lethals from the condition in *biennis*, but of course this cannot be assumed.

The form came to me through seed from plants which I saw growing in the University Botanic Garden, Cambridge, England in 1925. The Director of the garden, H. GILBERT CARTER, could tell me nothing of the history of the plants which may have been grown at Cambridge for a number of years. The form interested me because it differed from *biennis* in having stems red papillate and becoming light red below, midveins light red, broken streaks of red on sepals, and ovaries with red papillae (few). Because of this general development of anthocyan pigmentation, the plant has been carried in my cultures under the nickname *biennis red*.

My first culture of the plant was grown in 1928 from a collection of seeds sent to me from Cambridge. Since pollination takes place in the bud, cross fertilization is unlikely, and it was not surprising that this first culture (26.18) of 163 plants from 169 seedlings was uniform; the seed germination was 66.3 percent. From this point the race has been carried forward in a selfed line with a culture of 132 plants in 1927 and small cultures of 25–30 plants thereafter through a total of 11 generations from selfed seed. There have been no mutants in my cultures, *cantabrigiana* apparently being very stable, as is true of *biennis*.

Seed germination has been tested four times during the history of the line, the records ranging from 84.3 to 92.9 percent, a high ratio of seed fertility similar to that of *biennis*. Pollen is less than 50 percent good, *biennis* presenting a similar percentage. An examination of chromosome arrangement at diakinesis in the pollen mother cell (plant 35.18-2) showed circles or chains of ten and of four (figure 3) in contrast to the arrangement of circles or chains of eight and of six, characteristic of *biennis*.

I became aware of the position of the gamete lethals, the reverse of that in *biennis*, when in 1936–37 I grew reciprocal crosses between *cantabrigiana* and the homozygous species *franciscana*, *H[illegible]eri* and *blandina*. The re-

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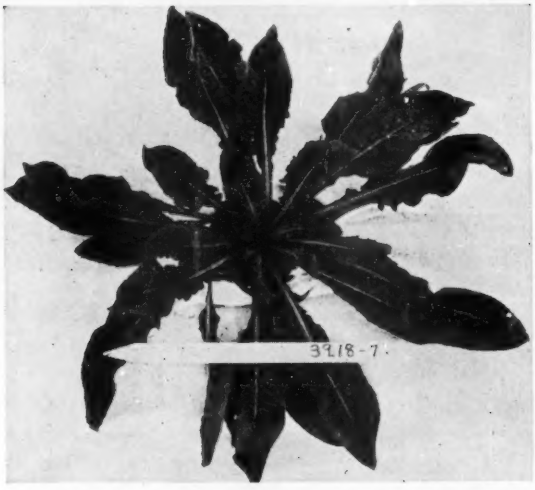

FIGURE 1.—Rosette of *Oenothera cantabrigiana*.

ciprocals resemble the Laeta and Velutina types described by DE VRIES. When *cantabrigiana* supplies the egg, the F_1 hybrid is Laeta since a rubens-like complex enters from the egg. When the pollen is from *cantabrigiana* the F_1 is Velutina because the pollen carries an albicans-like complex. The same three homozygous species crossed to *biennis* give Laeta when *biennis* as the pollen parent supplies the rubens complex and Velutina when *biennis* gives the egg with the albicans complex.

The contrasts between the Laeta and Velutina types were clear and consistent and the chief peculiarities in which there was complete agreement are given below:

	LAETA (Egg, rubens red, from *cantabrigiana*)	VELUTINA (Pollen, albicans red, from *cantabrigiana*)
Rosette leaves	Broader (6–7 cm), much crinkled. Midveins red.	Narrower (4–5 cm), somewhat crinkled. Midveins white.
Mature plant	Stems medium red papillate. Midveins light red. Leaves at base of plant broad (5–6 cm) strongly crinkled.	Stems strongly red papillate. Midveins white. Leaves at base of plant narrower (4–5 cm) almost plane.
Sepal tips	Slender, acute pointed, tendency to spread.	Thicker, blunt pointed, appressed.

With respect to morphological characters the contrast between bud tips is the most striking. The rubens-like complex in the egg of *cantabrigiana* brings into the Laeta type of hybrids the slender, pointed sepal tips with spreading tendencies characteristic of *biennis*. In the absence of the rubens-like complex, when *cantabrigiana* through the pollen introduces the albicans-like complex, the Velutina types of hybrids present sepal tips thicker, blunt, and appressed as found in *franciscana*, *Hookeri*, and *blandina*.

Reciprocal crosses of *cantabrigiana* to *franciscana* and *Hookeri* differ from corresponding crosses of *biennis* in one important respect: there are no classes of etiolated seedlings and rosettes which die early or produce weak plants. The cross *franciscana* by *biennis* gives a very large class of such etiolated seedlings and rosettes in sharp contrast to the few green and very vigorous plants that live. In the cross *Hookeri* by *biennis*, the seedlings are green, but the rosettes develop marked etiolation which gradually disappears in most of the plants under favorable cultivation. Corresponding crosses when *cantabrigiana* is involved are fully green and vigorous.

Description of *Oenothera cantabrigiana*

Mature rosette (Figure 1)	4–4.5 dm broad, dense, dark green. Leaves broadly elliptical, crinkled, 20–25 cm long, 5.5–6 cm broad. Midveins light red.

FIGURE 2.—Mature plant of *Oenothera cantabrigiana*.

Mature plant (Figure 2)	Central shoot 5–6 dm. Long side shoots from rosette, 5–6 dm, give a sprawling habit of growth. Stems red papillate, becoming light red below, pilose and puberulent the long hairs from the red papillae.
Foliage	Leaves at base of central shoot 17–18 cm long, 4 cm broad, somewhat crinkled. Leaves above broadly lanceolate. Midveins reddish.
Inflorescence	Bracts ½ to ⅓ length of buds.
Mature buds	6–6.5 cm long. Cone 2.3 cm. Sepals with broken streaks of red, pilose and puberulent, no red papillae. Sepal tips 3–4 mm long, slender, acute pointed, spreading. Hypanthium green.
Petals	2–2.3 cm long, golden yellow.
Stigma lobes	4 mm long, about 3 mm below tips of anthers.
Ovaries	Green with a few red papillae.
Capsules	20–23 mm long, green.
Pollen	35–40 percent good.
Seeds	Germination about 90 percent.

Chromosome configuration at diakinesis, circles or chains of ten, and of four chromosomes.

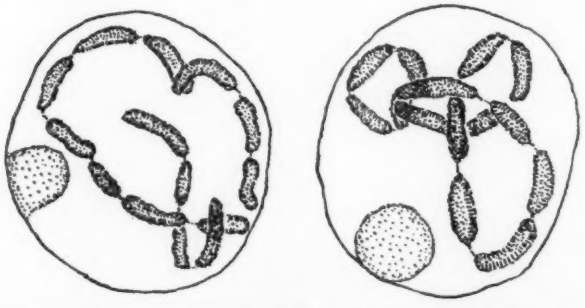

FIGURE 3.—Chromosome configurations of *Oenothera cantabrigiana*, circles or chains of 10 and of 4 chromosomes.

The discovery of the lethal situation in *cantabrigiana* has opened the possibility of obtaining two much desired plants for Oenothera genetics (1) a form close to rubens·rubens and (2) a form close to albicans·albicans. Particularly desired is rubens·rubens for the rubens complex of *biennis* has some peculiarities of genetical behavior that are not understood. At present, I am writing *cantabrigiana* as rubens red·albicans red. The pollination of *cantabrigiana* (egg, rubens red) by *biennis* (pollen, rubens) gives in the hybrid rubens red·rubens. Conversely, *biennis* (egg albicans)

pollinated by *cantabrigiana* (pollen albicans red) gives the hybrid albicans · albicans red.

These crosses have been made and the synthesized forms rubens red · rubens and albicans · albicans red have been carried into an F_2 generation. Also, certain interesting back crosses of the F_1 generations have been made. Cytological situations are being studied.

SUMMARY

Oenothera cantabrigiana is a structural hybrid closely related to *O. biennis* but with pollen and egg lethals reversed in position. A rubens-like complex is carried by the egg and an albicans-like complex by the sperm. Consequently, *cantabrigiana* crossed to the homozygous *franciscana*, *Hookeri* and *blandina* gives the Laeta type of hybrid when it furnishes the egg and the Velutina type when it supplies the pollen.

THE DISTRIBUTION OF INVERSIONS IN TRADESCANTIA

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Received March 29, 1940

THE comparative study of the cytology of species-hybrids has been a particularly fruitful one. Such analyses, dealing mostly with sterility determinations, chiasma frequencies, and chromosome configurations, have served as critical evidence for the establishment of probable interspecific, and even intergeneric, relationships, and simultaneously, have led to a better understanding of the methods by which isolation and subsequent speciation have taken place in the past, and are taking place at present. The justification for this method of approach to the whole problem of inter-relationships rests upon the assumption that the conjugation of parental chromosomes at meiosis is a relatively valid criterion of chromosome homology.

Since the advent of the salivary gland technique in Diptera, and more particularly, since the work of DOBZHANSKY (1937) and others on intra- and inter-specific hybrids in Drosophila, it has become increasingly evident that a considerable number of structural rearrangements are present which probably would never be detected in ordinary meiotic preparations. The changes are too small for legitimate pairing and crossing over to take place, and hence their presence is not revealed by the usual cytological techniques. Similarly, the regular and constant meiotic behavior in organisms with partial or complete chiasma localization may obscure profound structural changes which have occurred in genoms of the various species, and a failure to detect these differences in the hybrids is therefore no proof that such conditions do not exist. The inadequacy of cytological methods for the resolution of small structural changes when applied to plant material emphasizes the need of caution in the interpretation of relationships based on pairing in hybrids, especially those of the first generation where lower chiasma frequencies than those found in the parents are apt to be the rule. This we have found to be well substantiated by the present study.

One of the most frequently encountered meiotic aberrations in both pure and hybrid populations is that in which a portion of a chromosome is present in an inverted state. In plants this structural heterozygosity can be detected by chromatin bridges at anaphase I and II, and by the characteristic loop pairing at pachytene. In their extensive work on Tradescantia, ANDERSON and SAX (1936) conclude on the basis of chromosome behavior in F_1 hybrids that speciation in this genus has been primarily a function of gene change alone, and they make no mention of inversions

found either in the various species or in the species-hybrids which they studied. Later, SAX (1937) and DARLINGTON (1937) showed that inversions are present in all of the species observed. The frequency of this kind of structural change, as reported by them, was low with the one exception of a triploid *T. bracteata* Small investigated by SAX (1937). When the writer, in a meiotic survey of a group of diploid Tradescantia hybrids, found bridges in as high as 30 percent of the pollen mother cells of some plants, the present study was begun in an attempt to ascertain, if possible, the frequency of inversions, their general behavior and mode of transmission from one generation to another, and their possible rôle in the process of speciation within the genus.

The investigation presented in this paper, of which a preliminary report has been published (SWANSON 1940), has been a quantitative analysis of the meiotic behavior in Tradescantia insofar as it concerns inversions, chiasma frequencies, and chiasma localization. Studies have been made in the past on these three phases of meiotic activity, but they generally have been treated as separate entities. It is evident that while these three phases are superficially independent of each other, and can be treated as separate units of behaviour, there exists an interrelationship which is fundamentally important. From the results presented, it will be evident that the data obtained from the parental plants do not provide accurate information as to the kind and degree of structural change which exists between the chromosome complements of the contributing species. This is the result of a low chiasma frequency in the parental plants, and a restriction of crossing over to regions possessing little structural change. A shift in the location of crossing over toward the centromere as well as an increased range in the amount of crossing over by the segregation and subsequent recombination of genetic factors governing meiotic behavior has enabled us to reveal by a study of meiosis in the offspring, the presence of a greater degree of chromatin rearrangement than had been previously realized in this particular genus.

MATERIAL AND METHODS

The plants used in this study are two natural hybrids, called "Oak Hill 5" and "Oak Hill 13," and a number of offspring resulting from reciprocal crosses made between them. The parental plants were originally collected near Austin, Texas, and represent natural hybrids between *Tradescantia canaliculata* Rafin. × *Tradescantia humilis* Rose. They are intermediate in morphological characteristics with respect to their suspected parents. Collection and identification of these plants was made by DR. EDGAR ANDERSON of the Missouri Botanical Garden. More complete information on these species-hybrids may be obtained from ANDERSON and WOODSON'S (1935)

monograph of the genus. The Oak Hill plants are probably not strictly F_1 hybrids, since considerable back-crossing to one parent or the other may have occurred in the field and therefore their offspring do not represent a strictly F_2 generation, but it is to be emphasized that the offspring show considerable variability in growth habit, pollen sterility, and meiotic behaviour. Segregation has clearly taken place. Since the exact lineage of the Oak Hill plants can only be surmised it will be advisable to refer to them as "parental" plants, and to their offspring, collectively, as a "hybrid population."

The hybrid population composed a group of over 400 plants, only 28 of which were used in this study. Sterility determinations were made on the basis of full versus empty pollen grains, although germination tests in some cases indicate that many grains of normal appearance are nonfunctional. Chiasma frequencies were determined only from metaphase configurations, and all chiasma were classified as either terminal or interstitial, although the interstitial chiasmata are variable as to their location. Only strictly terminal chiasmata were classified as such. In scoring the number of pollen mother cells showing inversion bridges in both the parental and hybrid plants, it should be stated that only anaphase I configurations were counted, although bridges were also noticed frequently at anaphase II. The reason for disregarding anaphase II bridges in computing the percentages is that it has been assumed, not without evidence, that the relative bridge frequencies in the different plants would remain unchanged, since in a plant with a high bridge frequency at anaphase I, the frequency at anaphase II would be proportionately high, while a low anaphase II count would be found in a plant with a low anaphase I frequency. Chiasma and bridge frequencies for each plant were usually obtained from a single slide since environmental conditions were found to influence both of these to a considerable extent.

Meiosis in the parental plants

The general meiotic behaviour in Oak Hill 5 and 13 was essentially the same, and, with the exception of inversions, differed little from the behaviour of the Oak Hill plants which had been previously reported by ANDERSON and SAX (1936, table V, pp. 451). Metaphase configurations showed the typical Tradescantia type of pairing, that is, the bivalents were in the form of either rings or rods, with most of the chiasmata in terminal positions, but showing occasional interstitial loci paired (Plate 1, figure A). Four chiasmata per bivalent was the highest number recorded from any one bivalent configuration, giving a chiasma frequency per bivalent of 1.80 and 1.83 for Oak Hill 5 and 13 respectively. ANDERSON and SAX give frequencies of 1.6, 1.7, and 1.9 for the three Oak Hill plants

which they investigated. Inversion bridges were observed in four percent of the pollen mother cells examined in each parent (Plate 1, figure F). No more than one bridge per cell was seen in any of the preparations. The usual acentric fragment was visible in some cells, while it was invisible in others. Univalents were recorded in 20 percent of the pollen mother cells of Oak Hill 5, while Oak Hill 13 had a slightly higher percentage (Plate 1, figures C, F). Univalent formation was attributed generally to asynapsis, although some configurations suggested a premature separation of certain bivalents. Usually only a single pair of univalents was to be observed in any one cell, indicating perhaps that the same pair of chromosomes was involved each time. The certainty of this, however, could not be ascertained. The most extreme variation between the two plants was in the number of interstitial chiasmata per cell, Oak Hill 13 having about double the number that found in Oak Hill 5 (table 1). The range in variation from one cell to another was likewise greater in Oak Hill 13, several cells out of the 50 examined having as many as four interstitial chiasmata, while the other had only one cell with as many as three interstitial chiasmata. A slight variation existed in the number of terminal chiasmata per cell. The pollen sterility was approximately the same in each plant.

Meiosis in the hybrid population

Of particular interest, and that to which most attention was directed was the unexpectedly wide variation in meiotic behaviour among the plants in the hybrid population. The data are presented in table 1.

The variation in the number of terminal chiasmata per cell was small. When chiasmata counts were made on successive days, as was done in several instances for the purpose of verifying previous figures, the values were nearly identical, and showed no significant deviation. The average number of terminal chiasmata for each plant is apparently more or less constant, and is not subject to environmental variation as is the number of interstitial chiasmata. One, two, or three rod bivalents were found in almost every cell, so that cells with the maximum of 12 terminal chiasmata formed only a small percentage of the total number. The variation in the number of chiasmata at interstitial loci, however, was considerable, plant 195 having as low an average as .4 interstitial chiasmata per cell, and plant 105 having as high an average as 4.18, a variation of the order of 10:1. Figures B and C (Plate 1) illustrate some of the metaphase configurations from the hybrid population. The plants with the lowest interstitial chiasma frequency, as plants 101, 195, and 299, were quite similar in meiotic behavour to that observed in the Oak Hill plants (Plate 1, figure A). Figures B and C (Plate 1) with eight and five interstitial chiasmata respectively are characteristic of those plants with the highest frequencies. This high

degree of interstitial exchange gives plant 105, for example, a total chiasma frequency of 14.7 per cell, or 2.45 per bivalent. There were never more than two chiasmata per chromosome arm, one terminal and one interstitial, or two interstitial.

TABLE 1

Data on bridge and chiasma frequencies. (Based on counts of at least 50 cells each.)

PLANT	PER CENT BRIDGES	INTERSTITIAL XTA/CELL	TERMINAL XTA/CELL	XTA/BIVALENT	PER CENT UNIVALENTS	PER CENT STERILITY
O.H. 5	4	.615	10.225	1.8	20	21
O.H. 13	4	1.24	9.75	1.83	26	17
101	2	.42	10.44	1.81	10	—
195	4	.4	9.52	1.50	0	very
299	4	.44	11.10	1.92	2	11
50	4.4	1.20	10.55	1.96	20	10
19	6	1.14	10.71	1.98	6	19
296	7	.91	10.32	1.87	—	24
307	7	1.06	10.06	1.85	—	13
325	8	.5	9.9	1.73	26	—
404	8	.977	10.27	1.87	—	30
257	8	1.1	9.96	1.84	—	—
251	8.53	1.96	9.8	1.96	30	13
164	10	1.03	10.18	1.85	—	15
348	10	1.20	10.56	1.96	—	10
374	11.7	1.69	10.36	2.00	11	20
302	12.2	1.38	10.97	2.06	0	15
301	13.2	1.22	10.22	1.90	—	42
255	14	1.68	11.04	2.12	2	24
42	14	2.44	10.08	2.09	12	22
223	15	2.08	9.52	1.93	—	29
265	15	2.26	9.70	1.99	—	—
220	17	2.64	9.64	2.05	—	26
359	18	2.62	10.58	2.20	—	—
382	18.24	2.65	9.97	2.08	—	—
308 (8/14/38)	20.9	2.75	10.97	2.29	—	15
308 (11/3/39)	15.6	2.56	10.91	2.24	0	—
397	21.21	2.96	10.11	2.18	—	—
105 (10/1/38)	23.2	3.42	10.26	2.28	—	—
105 (6/10/39)	32	4.18	10.52	2.45	10	17

The total chiasma frequencies of most of the hybrid population examined were higher than those of the parents, only two of them, plants 195 and 325, being lower. Four of the hybrids, plants 101, 195, 299, and 325, were lower than either parent in the number of interstitial chiasmata per cell. Previously reported chiasma frequencies of *T. humilis* and *T. canaliculata* (ANDERSON and SAX 1936), the supposed parents of the Oak Hill

plants, give *T. humilis* a frequency of 1.7 and 1.8 chiasmata per bivalent, and *T. canaliculata* 2.6 per bivalent. It would seem, therefore, that in the hybrid population frequencies lower than one parent were obtained, but that none reached that of the other parent. Realizing that the amount of germ plasm contributed by each species in the origin of the Oak Hill plants is questionable, since some backcrossing may have occurred, the comparison of chiasma frequencies is of little significance. There is no doubt, however, that the variation expressed by the hybrid population through its chiasma frequencies is the result of a segregation of genetic factors governing pairing relationships at meiotic prophase. The various recombinations are reflected in an increased range of interstitial chiasmata per cell in the hybrid population as compared to those found in the Oak Hill plants. The significance of this increased pairing will be brought out later.

Structural hybridity has been previously reported in this genus (DARLINGTON 1929, 1937, SAX 1937b). Considerable chromosome rearrangement was noted in the pollen mother cells of the hybrid population. Besides the occurrence of univalents (Plate 1, figure C), structural hybridity was evidenced through the frequent appearance of dicentric chromatids and acentric fragments at first anaphase. In none of the preparations studied were rings or chains of more than two chromosomes found, an indication that reciprocal interchanges involving the terminal ends of the chromosome arms played no rôle in the upset of meiotic regularity. Inversion bridges were found in all of the plants examined, the frequencies varying from two percent of the pollen mother cells in plant 101 to as high as 32 percent in plant 105. Most of the bridge percentages (table 1) were higher than those reported by either SAX (1937b) or DARLINGTON (1937) for diploid species and hybrids.

Generally only one bridge per cell was found (Plate 1, figures D, E, F). Occasional cells, particularly those from plants with high bridge frequencies, were found possessing two bridges (Plate 1, figure G). Several cells showed three bridges (Plate 1, figure H). These cells bearing more than one bridge were of a relatively low frequency. Only two out of about 2000 cells examined showed two bridges in the same chromosome arm (Plate 1, figure I). This could result from (1) a double crossover within a single inversion involving all four chromatids, (2) crossovers occurring in two adjacent inversions (figure 4), or (3) two crossovers in overlapping inversions (figure 5). The first condition necessitates a relatively long inversion, while the other two conditions necessitate the presence of two bridges of unequal length as well as two unequal fragments. The latter seems to be the case, for the bridge at the left (indicated by the arrow) is definitely more stretched out than the other, and so must have originated from a

more proximal inversion. Our data likewise seem to disprove the presence of long inversions in this group of Tradescantia.

A crossover within an inversion should produce at anaphase I a dicentric chromatid and an acentric fragment. The latter, lacking a centromere, possesses no means of being assured regular distribution. It remains free in the cytoplasm, usually in the vicinity of the bridge (Plate 1, figures G, J), but occasionally at the edge of the spindle (Plate 1, figure E). It seems significant and worthy of mention that more than half of the cells bearing bridges lacked the fragment (Plate 1, figure D, I). This phenomenon has been encountered by both SAX (1937b) and DARLINGTON (1937) in Tradescantia, and by STEBBINS and ELLERTON (1939) in Paeonia, although not in such a high percentage of cells. These investigators postulate the presence of small sub-terminal inversions, and this is apparently true in the present case, for the bridges are approximately double the length of a chromosome arm, and with a diameter comparable to that of a normal arm. It would seem that some underlying cause must be responsible for the disappearance of the fragment, for too overwhelming an amount of evidence has accumulated disproving terminal fusion of chromosomes under natural conditions. Without doubt some of the fragments are too small to be readily seen (Plate 1, figure E), while others are obscured by the other members of the complement. Fragments of all sizes have been observed, grading from those longer than a chromosome arm to fragments that approach the limits of microscopic resolution.

DESCRIPTION OF PLATE 1

Photographs of aceto-carmine smear preparations of meiotic configurations in Tradescantia. Ca. 1000×.

FIGURE A.—Meiotic metaphase. All chiasmata at terminal loci.

FIGURE B.—Meiotic metaphase. High interstitial chiasma frequency.

FIGURE C.—Meiotic metaphase. High interstitial chiasma frequency. Pair of univalents showing.

FIGURE D.—Meiotic anaphase. Inversion bridge minus the usual acentric fragment.

FIGURE E.—Meiotic anaphase. Inversion bridge. Tiny fragment indicated by arrow.

FIGURE F.—Meiotic anaphase. Inversion bridge with fragment attached to end of normal arm. Univalent dividing precociously.

FIGURE G.—Meiotic anaphase. Two inversion bridges of different lengths. Fragment from the left bridge has not been released as yet.

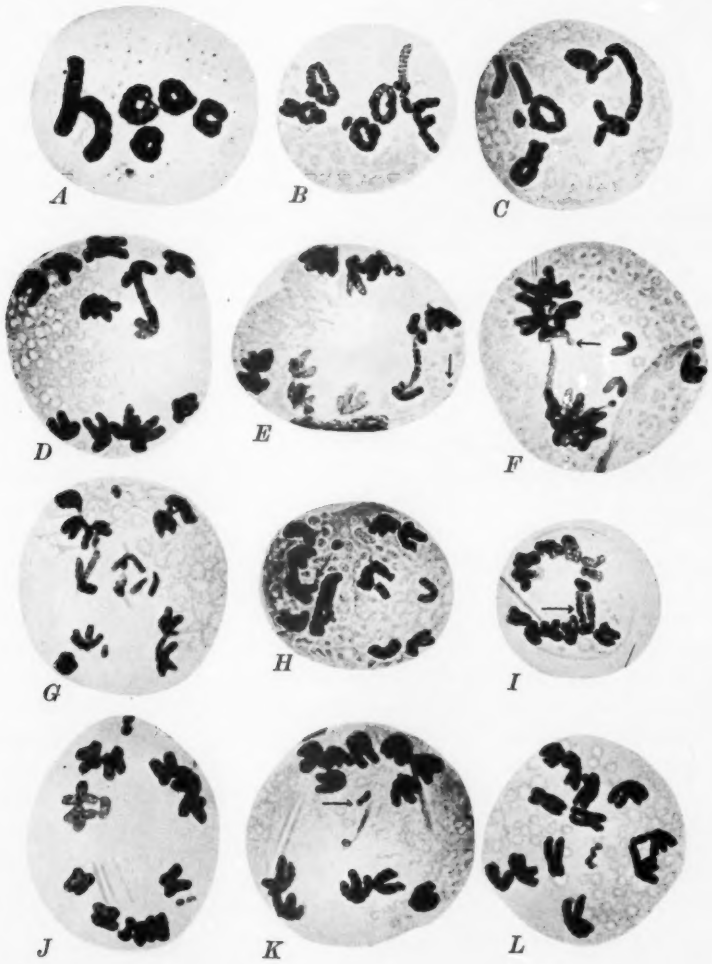
FIGURE H.—Meiotic anaphase. Three inversion bridges. Circular fragment visible (see text for explanation). Other fragment indicated by arrow.

FIGURE I.—Meiotic anaphase. Double inversion bridge. See text for explanation.

FIGURE J.—Meiotic anaphase from *T. paludosa*. Short inversion bridge and large fragment.

FIGURE K.—Meiotic anaphase from "Harvard" hybrid. Inversion bridge in the normal centric fragment. Acentric fragment indicated by arrow. Non-disjunction has resulted in a 5–7 distribution.

FIGURE L.—Meiotic anaphase from "Harvard" hybrid. Interstitial chiasma has delayed disjunction of the centric fragment.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The work of McCLINTOCK (1938) offers a likely explanation for this phenomenon. In numerous instances, she has likewise failed to find the fragment free in the cytoplasm. However, in occasional cells she has detected the fragment attached to the end of the normal chromatid by means of a chromatid thread, and by which it was pulled poleward to be included in the telophase nucleus, in which case it would probably be lost in the cytoplasm at the second meiotic division. McCLINTOCK has postulated the probable occurrence of a chiasma between the inversion and the end of the chromosome, this chiasma tying the fragment to the normal arm. DARLINGTON (1937) states that in Tradescantia the fragment is "attracted" to the end of the sister chromatid, but this seems unlikely.

With considerable terminal association present in Tradescantia, the absence of the fragment can be easily explained in some instances by the terminal attachment of fragment and normal arm, and such configurations (Plate 1, figures F, K) have been frequently encountered, although the nature of the union could not be detected. The fragments are indicated by arrows. Furthermore, as stated above, fragments so small as to be almost at the limits of resolution have been found in some cells. Union of such fragments with the end of a normal chromatid would probably allow the acentric portion to pass to the pole unnoticed, its minuteness not appreciably lengthening the normal arm of the chromosome. The possibility of a chiasma distal to the inversion or inversions giving rise to the extremely small fragments seems remote since the pairing length would undoubtedly be limited, but no other explanation is at hand unless a matrical binding of some sort is postulated.

Numerous fragments were visible and, on measurement, showed considerable variation in length as well as in diameter. It should be stated that the use of diameter and length measurements of fragment size is not a dependable method for distinguishing one fragment from another, and thereby estimating the number of inversions present in a particular plant. The degree of contraction of the fragments is extremely variable, some remaining quite stretched out as indicated by the width between adjacent coils, while others round into spheres of chromatin. This is especially true when using acetic acid-alcohol fixatives. It is possible therefore only to distinguish between fragments of distinct size differences.

All evidence to date points to the fact that the size of the fragment from any one inversion is constant in length no matter at what locus within the inversion the crossover occurs. With this in mind, one is led to the conclusion that numerous small inversions have arisen throughout the interstitial regions of the chromosome arms, particularly in the sub-terminal regions. This is borne out by the high frequency of bridges with small fragments or no fragments at all. Small sub-terminal inversions

would be expected to produce crossovers very infrequently, but with numerous inversions of this kind, the total amount of crossing over within them would approximate the figure which might be expected from a much longer inversion. The infrequent appearance of long fragments rules out the presence of a long inversion in the regions of most frequent crossing over. Longer fragments do appear more often in those plants with a higher chiasma frequency, but this has been interpreted as resulting from an occasional crossover in a short inversion close to the centromere. The infre-

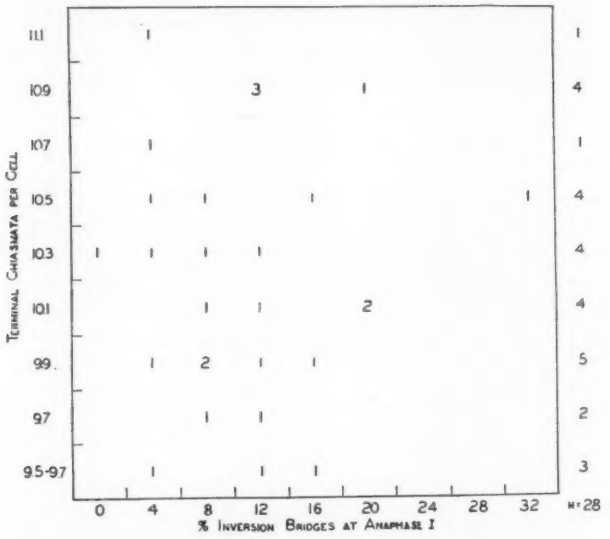

FIGURE 1.—Correlation between the frequency of terminal chiasmata per cell and the percentage of inversion bridges in the plants of the hybrid population. $r = .07 \pm .18$.

quent occurrence of a double bridge in a single arm (Plate 1, figure I) also militates against the presence of any long inversions.

The circular fragment found on one of the cells (Plate 1, figure H) is of interest. It could arise only from 2-strand double crossovers in adjacent (figure 4) or overlapping inversions (figure 5). This would release a circular acentric fragment as well as the usual straight acentric fragment (indicated by the arrow), which can be faintly seen in this figure attached to the lower normal arm of the extreme left-hand bridge.

If the sub-terminal and interstitial regions of the chromosome arms possess much inversion hybridity, and if the inversions are short and numerous rather than few and of random length, an additional inference can be made to the effect that an increase in crossing over in the total num-

ber of bivalents should be closely correlated with an increase in the percentage of cells showing inversion bridges. First it may be pointed out that no relationship exists between the number of terminal chiasmata and the number of interstitial chiasmata per cell, for if plotted against each other, a correlation coefficient approaching zero ($r = .043 \pm .19$) is obtained. A similar low value is obtained when the number of terminal chiasmata per cell is plotted against the percentage of inversion bridges (figure I). It may be concluded from this that the number of terminal chiasmata

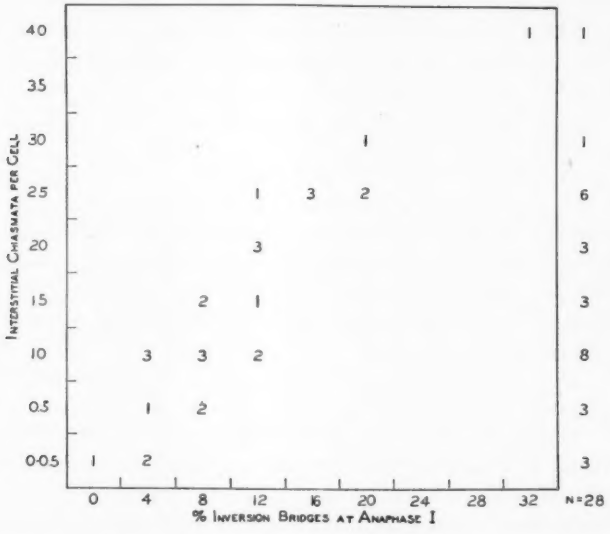

FIGURE 2.—Correlation between the frequency of interstitial chiasmata per cell and the percentage of inversion bridges in the plants of the hybrid population.

per cell approaches a value more or less constant for the genus regardless of the structural differences found in the interstitial regions of the chromosome arms, with such variation as might be expected from plant to plant.

If, on the other hand, the number of interstitial chiasmata per cell is plotted against the percentage of bridges, a high correlation ($r = .93 \pm .031$) is found (figure 2). This almost linear relationship indicates that the structural differences are equally numerous and probably quite similar in all the plants of the hybrid population, their ultimate expression being wholly dependent upon the particular chiasma frequency characteristic of the plant in question.

The occurrence of univalents in both the Oak Hill plants and in the

hybrid population was frequent (Plate 1, figure C, F). From what few observations were made as to the distribution and behavior of the univalents, they seemed to follow no regular course, although a good portion of them apparently reach either one pole or the other. Figure K and L (Plate 1) are cells from another plant, but they illustrate the unequal distribution of the chromosomes resulting from univalent formation. Rarely, the univalents were seen to divide equationally after the other paired chromosomes had passed to the poles (Plate 1, figure F).

The percentage of univalents varied greatly, among the fifteen plants examined for this irregularity, their frequencies ranging from zero to 30 percent (table 1). Since univalents result from an absence of chiasmata at metaphase, or from a premature separation of paired homologues, we should expect to find a fair negative correlation between chiasma frequency and the percentage of univalents. No such simple relationship is indicated by our data. STEBBINS (1938) has likewise pointed this out in Paeonia. MATHER (1936) has made it clear that in some plants crossing over in one bivalent is not entirely independent of crossing over in other bivalents in the same nucleus. Unfortunately, the individual frequencies for each bivalent cannot be obtained because of their close morphological similarity, but there does seem to be a tendency to maintain a certain level of chiasma formation, indicating that an increase occurs in the remaining bivalents if one of their number is unpaired. In the Oak Hill plants where univalent frequencies are high, it would seem that structural hybridity is the immediate cause of the unpaired condition. If, however, a comparison of these plants is made with such of the hybrids as plants 302 and 308 where bridge frequencies are high and no univalents are found in any of the cells, the more likely explanation is that a physiological incompatibility due to gene unbalance expresses itself by lowering the chiasma frequency, and that structural differences play but a secondary part in univalent formation. This would not apply to plants 42 and 105 where high chiasma frequencies are still accompanied by frequent univalents.

Physiological factors contributing to unbalance are likewise indicated in the sterility counts (table 1). In general, an increase in sterility follows an increase in the percentage of meiotic abnormalities, but the degree of sterility was always higher than could be accounted for on the basis of observed irregularities. Presumably certain chromosome combinations produce a gene unbalance as ANDERSON and SAX (1936) have suggested, these combinations being lethal in the haploid state. It is possible that undetectable interchanges occur in the interstitial regions giving rise to duplication and deficiency when in certain combination. The rôle of environmental fluctuations are known to affect pollen sterility to a marked degree,

even in pure species of Tradescantia (ANDERSON and SAX 1936), and in hybrid plants where physiological unbalance is undoubtedly present to some extent, environmental factors are probably even more effective in causing meiotic upsets than in the more stable types. Pollen fertility of Oak Hill 5 and 13 was high, being 79 percent and 83 percent respectively. This is a good deal higher than the fertility of the majority of the species-hybrids reported by ANDERSON and SAX (1936), and is quite possibly an indication that Oak Hill plants are not strictly F_1 hybrids, but that some backcrossing to the parent plants has increased the fertility.

Experimental data

The direct influence of the environment on chiasma frequency is indicated by two sets of data collected from plants 105 and 308 at different times (table 1). The variations are slight, but significant, since a correlated deviation is also seen in the percentage of inversion bridges. Within a physiological range, warmer temperatures seem to favor chiasma formation, particularly in the interstitial regions of the chromosome arms. To test this under controlled conditions, plants of a clonal line derived from a plant of known high bridge frequency ("Harvard" hybrid of unknown origin) were placed in a warm chamber (27°C) and in a cool greenhouse (12–15°C), and the meiotic behavior was compared with data collected from the same plants grown at the normal greenhouse temperatures (19–22°C). The data are given in table 2. The high percentage of univalents is due to the presence of a segmental interchange involving a short terminal segment, as shown by the formation of chains and rings of four chromosomes. Low temperatures obviously hinder crossing over, and in this interchange hybrid, the number of trivalents and quadrivalents is greatly reduced. It is evident that while physiologically favorable temperatures cannot completely overcome the lack of pairing conditioned by structural hybridity, such temperatures have an influential effect on pairing factors such that the interstitial regions are given greater opportunity to crossover. In the "Harvard" hybrid, as in the hybrid population, this

TABLE 2

Data on Harvard hybrid grown at different temperatures.

TEMP.	n	INTERSTITIAL XTA/CELL	TERMINAL XTA/CELL	PER CENT UNIVALENTS	PER CENT BRIDGES
12–15°C	70	1.6	6.07	78.5	17.5
19–22°	90	2.88	7.7	54.	26.1
27°		3.88	7.62	53.	47.8

increased crossover frequency in interstitial regions results in a marked increase in the bridge frequency.

The behavior of the so-called normal centric fragment found in Tradescantia is of interest. Although their origin is in doubt, the fragments are undoubtedly homologous with portions of the major chromosomes, for pairing with them does occur (DARLINGTON 1937). Such pairing has been occasionally seen in our material. All of the fragments found in this genus are not homologous with each other, nor are they morphologically alike. WHITAKER (1936) states that the kind found in *T. paludosa* Anders. and Woodson has a terminal centromere, and our observations bear this out (Plate 1, figure J). Figures K and L (Plate 1) taken from the "Harvard" hybrid show clearly in the bridge complexes that the fragment here has a medianly placed centromere. Interstitial chiasmata were formed in this small chromosome (Plate 1, figure L), interfering with its normal anaphase movement in such a way that it lags behind the major chromosomes, whereas it usually divides along with the others (Plate 1, figures G, J). The fragment, like the major chromosomes, possesses an inversion, and, rarely, forms an inversion bridge (Plate 1, figure K). The acentric fragment is being pulled poleward by its attachment to the arm of the upper normal sister chromatid (fragment indicated by arrow).

DISCUSSION

Meiosis in Tradescantia

Metaphase configurations in this genus are chiefly seen as ring and rod bivalents, with approximately 80 percent of all associations at terminal loci. Since pre-metaphase behavior cannot be studied favorably because of the difficulty of obtaining good preparations, earlier behavior must be deduced from metaphase configurations.

Pairing in Tradescantia is initiated at the distal ends of the chromosome arms, and proceeds from there in proximal direction. The reasons for this are not well known. WHITAKER (1936) suggests that strong polarization favors the initiation of distal pairing. However, other organisms in which pairing is initiated at the centromere are similarly polarized, so we can conclude that polarization alone is not the determining factor. It is quite possible that structural heterozygosity, due to inversions and internal non-homologies, is so great that pairing is delayed interstitially, permitting the relatively free homologous ends to come into paired alignment first. The evidence for distal pairing is both direct and indirect. Indirectly, the frequent occurrence of proximal interlocking of bivalents (ANDERSON and SAX 1936), the pairing behavior of fragment chromosomes, and the variation in the number of interstitial chiasmata as contrasted with the relative constancy in the number of terminal chiasmata are best interpreted by

assuming an early pairing of ends which permits terminal association, but which need not necessarily extend into interstitial regions. Direct observations have been made by NEBEL and RUTTLE (1936) revealing that, except for the occasional pairing of ends, very little homologous union has occurred. A strikingly parallel situation is found in Paeonia (STEBBINS and ELLERTON 1939).

It may be argued that this prevalence of termial chiasmata is a function of a high degree of terminalization as it is in some plants as Campanula. DARLINGTON (1929) has made diplotene observations indicating some movement of chiasmata toward the ends of the chromosome arms. The type of coiling in Tradescantia (SAX and HUMPHREY 1934) would seem to invalidate DARLINGTON'S observations, for no reversals in the direction of coiling were to be found as would be expected in 50 percent of the cases in which a chiasma had been formed, and was then terminalized. This datum would itself be invalidated were coiling initiated after the occurrence of crossing over, as it might well be. Two other arguments might be advanced. It may seem logical to postulate that the frequency of crossing over is similar in all of the plants and that the variation in the number of interstitial chiasmata per cell is but an expression of a variation in the degree of terminalization, that is, terminalization may be partial or complete depending on the time at which metaphase came in, whether early or late. Or again, the degree of terminalization may be looked upon as a function of structural heterozygosity, since the more heterozygous a plant is for inversions, the greater would be the obstruction to the terminalization of any proximal chiasmata which had been formed previously. Two bits of evidence, however, seem to leave little doubt that the amount of crossing over, and, hence, the chiasma frequency, is genetically determined. Firstly, the high correlation between interstitial chiasmata and bridge percentage (figure 2), and secondly, the correlation of temperature with an increase in chiasmata and bridge percentage point indubitably to a variation in crossing over and not to a variation in the degree of terminalization or a difference in structural heterozygosity. So while we recognize that some terminalization must take place since all chiasmata must necessarily have their origin interstitially, the movement necessary to reach the end of an arm is slight, and affects only those chiasmata lying in extreme distal portions. MATHER'S (1940) recent data are of interest in this respect, for he shows that the movement of chiasmata is apparently an all-or-none reaction, especially as regards interstitial chiasmata.

In order to evaluate the significance of chiasma localization in structural heterozygotes, it will be well to consider briefly how crossing over in Tradescantia is controlled. This control is exercised through at least three factors: (1) environment, (2) genetic factors, and (3) structural changes.

The first two are presumably operative in all organisms, while the influence of the last is dependent upon the kind and degree of structural differentiation present at any one time. All three factors exert an active control in Tradescantia.

The effect of environmental factors in this genus has been adequately demonstrated and discussed here and elsewhere (SAX 1937a). The evidence we have obtained in this instance is in partial accord with WHITE's (1934) observations in the Acridiidae, where he showed that at the extremes of temperature the chiasma frequency showed significant increases. Our obobservations in Tradescantia (table 2) differ in that at the low temperatures a considerable decrease in chiasma frequency, and a large increase in univalent formation, took place. Other evidence (SWANSON unpub.) seems to show that above 30°C a similar lowering of chiasma frequency is to be found, thus giving a unimodal curve instead of one with two maxima as WHITE obtains, and as PLOUGH (cf. WHITE 1934) has demonstrated for crossing over in Drosophila. STEBBINS (1938) postulates cooler temperatures as a factor in lowering the frequencies he observed in Paeonia. It may well be, as MATHER (1936b) contends, that any factor influencing crossing over will have its greatest effect in the vicinity of the centromere, and not at the ends of the chromosome arms. It is quite probable that this localization of effect may be due in part to the presence of heterochromatic portions in the regions involved (MATHER 1939), although no cytological proof of this in Tradescantia is at hand.

The influence of structural hybridity on crossing over is obvious. The introduction of any change in the linear arrangement of the genes reduces the possibilities of pairing, and may so interfere that by the time the ends have paired, for example, as in Tradescantia and the New World peonies, the interstitial regions will have passed through their division cycle, and will be in a condition prohibitive to crossing over. STURTEVANT and BEADLE (1938) show that in Drosophila, where pairing and crossing over originate at the centromere and proceed in a distal direction, an inversion exerts its greatest unfavorable influence—that is, it prevents the pairing of adjacent homologous portions—in regions distal to it. In Tradescantia, where pairing originates distally, the disruptive influences might be expected to be felt more in the regions proximal to the inverted portion. The fact that considerable structural differentiation is present in the hybrids, however, does not adequately explain the variation in chiasma frequency, as has been pointed out above. More likely, as an explanation is the fact that the individual chiasma frequencies are but the visible expression of certain combinations of genes governing meiotic behavior. Genic combinations favoring increased chiasma formation apparently so alter conditions at the time of crossing over that the regions of crossover frequency are

shifted toward the centromere, thus including portions of the chromosome arms which previously, in the Oak Hill plants, were undisturbed insofar as crossing over was concerned. The relatively inverted regions are provided with a greater opportunity for legitimate pairing, increasing the bridge frequency, and simultaneously increasing the number of interstitial chiasmata at metaphase, since all chiasmata within inversions as well as proximal to inversions would have difficulty in becoming terminalized. It seems clear, then, that an active genetic control of chiasma formation is present in Tradescantia, with structural hybridity playing a secondary rôle. PETO (1934) has previously postulated a genetic control of chiasma frequency in the Festuca-Lolium hybrids, while LAMM (1936) suggests that several basic genes, together with modifiers, could account for the difference in chiasma frequency observed in inbred rye.

Some difficulty is experienced in attempting to visualize clearly the exact manner in which the genetic control of meiosis is exercised. MATHER (1936) has shown in Drosophila that a temporal sequence in chiasma formation is present, the first formed chiasma being adjacent to the centromere, and then the succeeding chiasmata being formed distal to it. The centromere seems to be the determining mechanism in this case. In Tradescantia, with distal pairing and evidence of incomplete synapsis (NEBEL and RUTTLE 1936), it seems probable that a variation exists in the total paired length of the chromosomes at the time of crossing over. Possibly a time-limit to pairing is in effect. Since crossing over can only occur between paired homologues, the amount of crossing over, and hence chiasma formation, reflects the total length of the paired portions. In plants with a low chiasma frequency, the amount of pairing just reaches the threshold value necessary for sufficient chiasma formation to effect a regular meiotic distribution of the chromosomes.

It is evident that with chiasmata localized at the homologous distal ends, few meiotic irregularities appear. This is generally the case in the hybrid plants with a low chiasma frequency. On the basis of meiotic behavior of F_1 hybrids, ANDERSON and SAX (1936) have stated that there is little evidence of structural change in the complements of the different species of Tradescantia. From what we know now, this meiotic regularity in the first generation is to be expected, since the F_1 plants usually possess a lower chiasma frequency than the parental plants, and inversions would be even less in evidence than in the parents. This is indicated by data on inversion bridges in *T. paludosa* Anders. and Woodson, and an F_1 hybrid with *T. paludosa* as one of the parents (SAX 1937b, table 2, p. 526). Speciation, as judged from such observations, is seemingly a matter of gene differentiation alone, and it can be inferred that an interpretation of species relationships in Tradescantia, and possibly in other genera, based on

chiasma frequencies and meiotic irregularities in the F_1 generation may be misleading. When this is true, the F_2 progeny must be utilized to reveal the correct status of interspecific relationships insofar as they may be determined cytologically.

Inversion hybridity

The occurrence of inversions both in plants and in animals has been well established by many investigators: MUNTZING (Crepis, 1934), RICHARDSON (Lilium, 1936), DARLINGTON (Orthoptera, 1936), DARLINGTON and GAIRDNER (Campanula, 1937), STEBBINS (1938) and STEBBINS and ELLERTON (1939) in Paeonia, and DOBZHANSKY (1937) and others in Drosophila. It is now taken for granted that most natural populations will reveal inversions, this being particularly true in clonal lines, and in cross-fertilized organisms. In the former category, there is apt to be a gradual accumulation of structural changes, none of which *per se* will have any consequence other than to initiate possible changes due to position effect. In the latter, the frequency of inversions will vary widely depending upon the rate of occurrence, the probability of becoming established, the rate of elimination, and numerous other factors, none of which are sufficiently well understood at the moment to be evaluated.

The evolutionary significance of inversions and their rôle in the process of speciation has received the special attention of DOBZHANSKY and others (DOBZHANSKY 1937, DOBZHANSKY and STURTEVANT 1938), working on *Drosophila pseudoobscura*. The third chromosome of this species has provided a wealth of material, and by means of the salivary technique, it has been possible to establish the probable race history of *D. pseudoobscura* on the Pacific Coast. The inversion, as pointed out by DOBZHANSKY (1937), becomes a focus of discontinuity, and an opportunity for incipient speciation is at hand since within this gene sequence mutations can arise which, on the basis of random chance, will be unlike any other mutations. The differentiation is thereby intensified. Favorable gene combinations included in the inversion will aid in its establishment, and possibly in the establishment of a new variant. That this, however, is a long time process is indicated by the fact that with the great variability of inversions in *D. pseudoobscura*, the species morphologically is remarkably uniform and has very little recognizable diversity of external form (DOBZHANSKY and STURTEVANT 1938).

The fate of an inversion, or a group of inversions, is dependent upon a number of factors. It is evident that a short inversion possesses a distinct advantage over longer ones with regard to survival value since, as a rule, the difficulty of pachytene pairing, and hence crossing over, is in inverse proportion to the length of the inversion. This does not hold for salivary

gland pairing, where HOOVER (1938) has shown that there is no correlation between length of inversion and the degree of synapsis, for all inversions, regardless of length, pair completely in about 80 percent of all cells. No data exist comparing salivary and pachytene pairing, but it would seem that pairing time would be the limiting factor at pachytene but not so in the salivary gland cells. The advantage possessed by any particular inversion in maintaining itself free from crossing over is doubled if another inversion lies in the immediate vicinity. Chiasma localization is of equal importance, for if crossing over is restricted to homologous uninverted regions, inversions are similarly protected from elimination. Single crossovers are eliminated (STURTEVANT and BEADLE 1936), and only two and three strand double crossovers are effective in causing recombination within an inversion. This demands a comparatively long inverted section. STURTEVANT and MATHER (1938) adequately discuss advantageous heterozygosity in respect to favorable gene sequences and heterosis effects as it relates to inversions.

The occurrence of inversions in pure species of Tradescantia is apparently widespread. SAX (1937b) reported their frequency in four species; DARLINGTON (1937) reports inversions in diploid, triploid and tetraploid plants of *T. virginiana* L. and *T. bracteata* Small; GILES (unpub.) has located several in the complement of *T. rosea* Ventenat. A study of two plants of *T. paludosa* Anders. and Woodson from different localities gave bridge frequencies of 3 percent and 5.18 percent, with a considerable variation in the size of the fragment. These figures approximate SAX's (1937b) data on the same species. By arbitrarily dividing the chromosome arms into three parts, distal, median, and proximal, and then measuring the length of the fragment, an approximation can be reached as to number and location of the inversions. Ten measurements from one of the *T. paludosa* plants gave one fragment as long as $\frac{1}{3}$ of an arm, seven as long as $\frac{2}{3}$ of an arm, and three as long or longer than the single arm. A variation in the diameter of the fragment was also noticed, and several other bridges lacked the fragment. There are, then, at least four, and probably more, inversions in the chromosomes of this plant, but their apparent small size, and the localization of chiasmata, precludes a high frequency of crossing over within any one of the inversions. Figure J (Plate 1) illustrates the large fragment and short bridge found in this plant, but which was not encountered in the other plant of this species, although their chiasma frequencies were similar. Evidently the inversions in the two plants are not identical in location and length. Data from a triploid *T. bracteata* (SAX 1937b) and from triploids of the same hybrid population considered in this study (GILES unpub.) similarly indicate numerous inversions, and as would be expected, the percentage of bridges is generally higher than that found in

diploids since the effective pairing length is greater in triploids than in diploids.

Mention has been made of the fact that a goodly number of the cells bearing bridges lack the usual acentric fragment. This, together with the fact that numerous tiny fragments were found, points unquestionably to small sub-terminal inversions. It is perhaps difficult to understand why these small inverted sections should pair at all, but the type of pairing found in Tradescantia, and the sub-terminal location of the inversions, offers a likely explanation for this seeming anomaly. McCLINTOCK (1933) showed that small inversions as a rule pair non-homologously, or not at all. The torsional force of 2-by-2 association often forces the non-homologous regions into a paired condition. This is in Zea where pairing presumably is initiated at the centromere. With terminal pairing, it is not difficult to imagine that the relatively inverted sub-terminal regions of the arms, being capable of greater movement than an interstitial region, are freer to pair legitimately without the necessity of forming the customary loop, leaving the homologous strictly terminal regions unpaired. If the sub-terminal inversion is greater in length than the homologous portions distal to it, then the forces of homology at the time of initial pairing would be stronger in the vicinity of the inverted region than they would be in the neighborhood of the homologous distal portions resulting in straight inversion pairing rather than loop pairing (figure 3). The arm on the other side

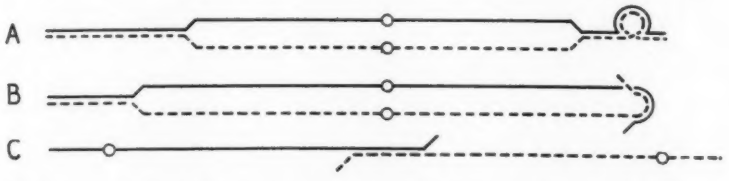

FIGURE 3.—Diagrammatic representation of how pairing can take place in short sub-terminal inversions where the inversion is longer than the uninverted region distal to it. Homologous chromosomes are shown as single rather than as double threads for the sake of clarity. *A* shows the conventional loop type of pachytene pairing. *B* and *C* show how inversion pairing can take place without the formation of a loop. See text for discussion.

of the centromere could move into position without hindrance, since polarization brings the arms relatively near to each other, or the bivalent could assume the form of a rod. The presence of a truly terminal inversion such as KAUFMANN (1936) has found in Drosophila seems to be ruled out in Tradescantia by the fact that in nearly every plant, occasional cells showed 12 terminal chiasmata at metaphase, a condition not possible were such a heterozygous region present.

The presence of more than a single inversion in at least one of the chro-

mosome arms of this group of hybrid plants has been mentioned (figure H and I, Plate 1). These two anaphase configurations have probably arisen from double crossovers in two adjacent inversions, or in overlapping inversions. The possible configurations resulting from such double crossovers are schematically presented in figures 4 and 5. The figure showing both the circular fragment as well as the customary straight fragment (figure H, Plate 1) probably resulted from a 2-strand double crossover at loci 1 and 2 (figure 4, 5). 3-strand double crossovers (1, 3, in figure 4, and 1,4, in fig-

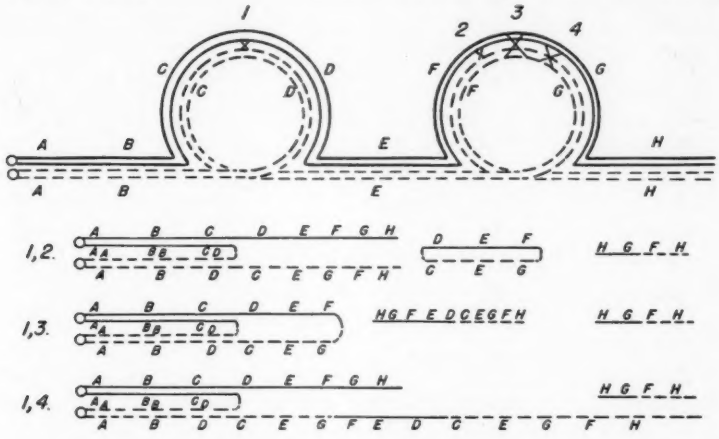

FIGURE 4.—Diagrammatic representation of crossing over in adjacent inversions. The loci at which crossing over takes place are designated by numbers. The chromatid configurations resulting from 2-, 3-, and 4-strand crossing over are given below.

ure 5) would give a double bridge configuration (Plate 1, figure I) with two acentric fragments. Unfortunately, neither fragment could be located although the unequal lengths of the two bridges leaves no doubt as to the correct interpretation. Configurations of the sort just mentioned have been previously described in Tulipa (UPCOTT 1937) where structural hybridity is extensive.

A configuration theoretically possible, but one which has not been observed, is that resulting from a 3-strand double crossover at loci 1 and 4 in adjacent inversions or at loci 1 and 3 in overlapping inversions. This type should occur with a frequency equal to that of either of the other two just described, but it is a type more easily overlooked at anaphase. It is evident from the diagrams that one of the free arms will contain a considerable portion of duplicated, and, in the case of adjacent inversions, of triplicated genic material. That is, the inverted portions of the chromosomes will be duplicated, and in an arm bearing two adjacent inversions, the re-

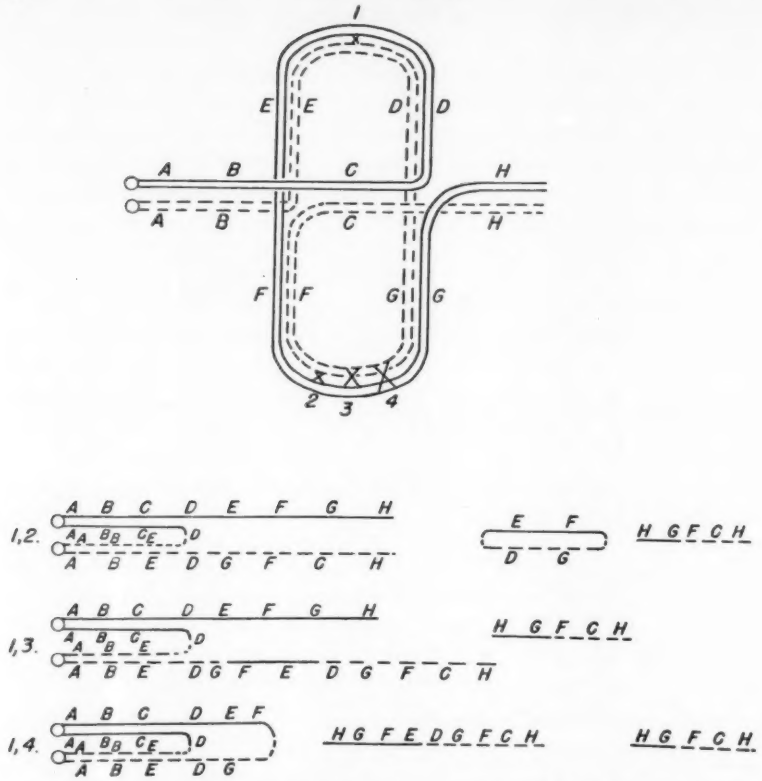
1
E E D D
A B C H
A B C H
F F G G
2 3 4
1,2.
A B C D E F G H
A B E D G F C H
E F
D G
H G F C H
1,3.
A B C D E F G H
A B E D G F E D G F C H
H G F C H
1,4.
A B C D E F
A B E D G
H G F E D G F C H
H G F C H

FIGURE 5.—Diagrammatic representation of crossing over in overlapping inversions. The loci at which crossing over takes place are designated by numbers. The chromatid configurations resulting from 2-, 3-, and 4-strand crossing over are given below.

gion between the inverted portions (region E in figure 4) will be in triplicate. In this manner we can see that inversions can act as a potential source of new chromosome types, although their ultimate survival and establishment is a moot question, since the addition of chromatin might readily lead to considerable unbalance and inviability.

A still further possibility for deriving new chromosome types may be demonstrated in figure 5. It is evident from this diagram that pairing in the *C* region presents an opportunity for crossing over. A single crossover in this region will produce two greatly altered chromatids. By following out the paired threads it will be seen that one chromatid will be extremely short, containing sections ABCH, whereas the other will be considerably lengthened and will consist of the regions ABEDGFCDEFGH provided

crossing over occurs at no other locus. Additional crossovers within the paired loops will greatly complicate the results. Starting with a chromosome possessing a median centromere, it is thus possible to derive two heterobrachial chromosomes, one having a sub-terminal centromere, and the other one sub-medianly placed. Although not known in the Virginiana Tradescantias, these chromosome types are found in other members of the genus, that is, *Tradescantia rosea*, as well as in other genera of the Commelinaceae (ANDERSON and SAX 1936), and while little is understood or known of the phylogenetic sequence within the family, the possibility of the derivation of new chromosome types through inversion crossovers should not be overlooked. There is likewise some evidence for the belief that the fragment chromosomes found commonly in Tradescantia might have a similar origin, for pairing of these fragments with major chromosomes has been seen to take place both at the terminal ends and in the region of the centromere. This is what would be expected if the interstitial regions were lost through some such mechanism as that postulated above, leaving a shortened arm on either side of the centromere. The relatively small amount of genic material added to the normal haploid complement by the retention of such a fragment would presumably contribute but little to meiotic irregularity or genic unbalance. This explanation of fragment origin is in better accord with the pairing relationships observed than is the alternative explanation of merely losing the distal portions of the chromosome arms, for with such an occurrence, no terminal pairing of the fragment would be possible. It may not be altogether coincidental that in Tulipa (UPCOTT 1937) and Fritillaria (FRANKEL 1937) where inversions are frequent and widespread, fragments are also common.

It has been previously mentioned that pollen sterility in the hybrid population is always higher than can be accounted for on the basis of observed irregularities at meiosis. Undoubtedly some mechanism contributing to genic unbalance in the haploid generation is responsible for the observed discrepancy. With numerous inversions present in Tradescantia, it seems not unlikely that some of the inversions may be of the included type (STURTEVANT 1938). This would be extremely difficult, if not impossible, to demonstrate cytologically in Tradescantia, but as STURTEVANT points out, structural heterozygote possessing chromosomes ABCDEFGH and AGCDEFBH—the latter the result of an inversion within an inversion—can pair and have crossing over take place in the CDEF region without bridge formation at anaphase, but that the chromatids involved will be both deficient and duplicated for certain genes. Pollen grains bearing these altered chromatids will be seen as empty grains at the time of maturity, since as GILES (unpub.) has shown, even small deficiencies are lethal before the microspore division has taken place.

From the data derived from the hybrid population we may infer (1) that the contributing species had become structurally differentiated in an independent fashion after their divergence from a common stock, and that this differentiation receives expression when the two genoms are brought together in the F_1 and later generations, or (2) that both parents possessed similar inversions which were established before divergence, and that genetic segregation had so shifted the location of crossing over that regions which up to this time had been more or less isolated were now undergoing a genetic shuffling. That the second is correct is indicated by the fact that all good species investigated to date have possessed inversions, but that the first inference is equally so is demonstrated by the considerable bridge frequency in a number of the plants in the hybrid population. In any event, the first has been shown to be dependent upon a favorable chiasma localization for visible expression at meiosis. It is very probable that in species which have become stabilized, an equilibrium is established to some extent between chiasma localization and inversions, such that inversions located in proximal regions can be carried on indefinitely without impairment to the species in question. Such plants as 101, 195, and 299, with a regular meiotic behavior, but with a low recombination rate as evidenced by a restriction of chiasma formation to the terminal regions, possess a selective advantage over those plants permitting crossing over to occur at random in the chromosome arms insofar as the maintenance of structural heterozygosity is concerned. Besides having a higher fertility, these plants also are in a better position to maintain favorable gene sequences (STURTEVANT and MATHER 1938) which might arise in the interstitial regions, for a low recombination rate will protect them from being broken up. This equilibrium, as we have seen, is upset in the F_2 generation due to genetic segregation and subsequent recombination of factors governing chiasma formation.

It is evident from figure 2 that there exists in the hybrid population an almost constant ratio between the number of interstitial chiasmata per cell and the percentage of inversion bridges, that is, for each increase of one interstitial chiasma there is an increase of approximately eight percent in the number of bridges. In other words, of every 12–15 chiasmata occurring at interstitial loci, one will be the result of a crossover within an inversion loop, and will express itself at anaphase as an inversion bridge. Figure 6 demonstrates this more clearly. Plants 325 and 50 depart markedly from this ratio, the former having a ratio of 6.2:1, and the latter a ratio of 27:1. This undoubtedly results from a greater structural heterozygosity in plant 325, and a greater structural homozygosity in plant 50 than is characteristic for the majority of the plants studied. For the group of plants as a whole, however, this ratio holds. The ratio, then, whether

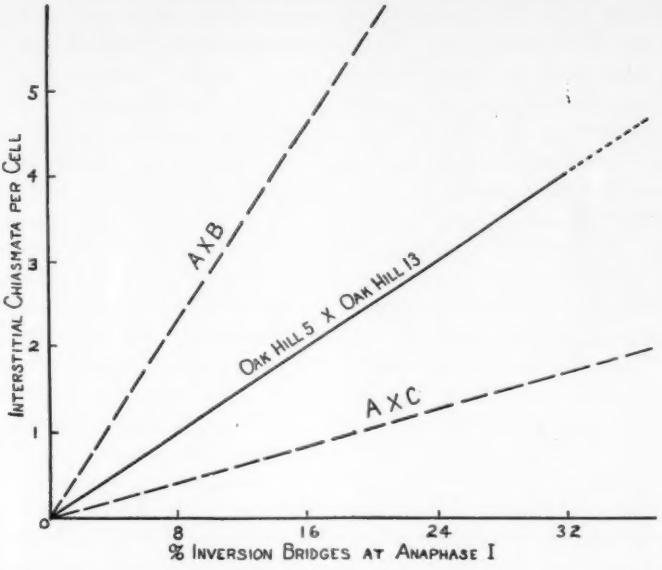

FIGURE 6.—Relation between interstitial chiasmata per cell and the percentage of inversion bridges in the offspring of the Oak Hill 5×Oak Hill 13 cross, as well as for two hypothetical crosses of Species A×B, and Species A×C. See text for discussion of the phylogenetic significance of such relationships.

high or low, has some phylogenetical significance, and it may possibly be of value in determining the degree of divergence of two species from a common stock. Thus, if species A were crossed on one hand with species B, and on the other with species C, and the ratios indicated by the curves in figure 6 were realized, one could conclude that, structurally, species B was more closely related to species A than was species C. The lower the ratio, the further the two species in question are separated, structurally, since a slight increase in the number of interstitial chiasmata per cell would raise the bridge percentage considerably. A high ratio would, on the other hand, indicate but little structural divergence, since with but few inversions, the chances of an interstitial chiasma occurring within an inversion loop would be greatly lessened since such loops would be of less frequent occurrence. It will be realized, of course, that this method of determining the relative inter-specific structural heterozygosity of a group of species has certain limitations: (1) the amount of intra-specific variation of this ratio must be rather thoroughly established before it can be of any value, (2) it can only be used where a large number of plants are available for cytological study since the ratio is determined for a group of plants, and not for single speci-

mens, and (3) it would probably be applicable only to such genera as Tradescantia, where inversions are small and numerous, and where factors governing meiotic pairing show segregation in the F_2 generation.

Of theoretical interest in Tradescantia is the fact that two conditions are found working in opposition to each other. These are (1) self-sterility, and (2) the presence of inversion with considerable chiasma localization. The first facilitates the recombination of genes responsible for variability, thus preventing genetic homozygosity; the second limits recombination, for unless two and three strand double crossovers occur, all inversions are either inherited as blocks of genes or are eliminated. HOOVER (1938) in a study of nine inversions of variable length in Drosophila finds that "cross over classes are completely suppressed within the limits of the inversion itself; furthermore, adjacent regions are strongly affected, the effect decreasing with distance from the aberration." The percentage of possible recombinations is therefore drastically lowered. In the hybrid population the barrier to recombination is somewhat overcome by increasing the limits within which crossing over can take place, thus including those regions which previously had enjoyed segmental isolation. The shuffling of gene blocks provides an opportunity for advantageous combinations to appear which, if provided with a means of perpetuation, will lead to variation and discontinuity within the group.

The presence of self-sterility in this genus undoubtedly accounts for the perpetuation of some of the structural heterozygosity. This phenomenon in Tradescantia agrees essentially with the oppositional factor hypothesis (ANDERSON and SAX 1934), in which it is postulated that pollen grains possessing *S1* and *S2* sterility genes will not send tubes down an *S1 S2* style, but will send tubes down an *S3 S4* style. If the sterility genes are located within an inversion, or in regions adjacent to an inversion, a mechanism is at hand to maintain such a structural heterozygosity from one generation to another. This, however, will account for only one inversion, and even stable species in this genus have more than this. Quite likely, in Tradescantia, is the possibility of a balanced lethal system such as is found in Rhoeo or Oenothera, with lethal genes being located in or adjacent to the inversions instead of being bound in with a segmental interchange complex. This hypothesis, however, awaits further analysis.

It has been pointed out previously (ANDERSON and SAX 1936) that within the *virginiana* group of Tradescantia the factors of importance in speciation have been genic differentiation, hybridization, and structural changes. These latter changes are fragmentation and segmental interchange. Evidence presented here, and by others (SAX 1937b, DARLINGTON 1937, GILES unpub.), makes it obvious that inversions must be looked upon as an equally if not more important evolutionary factor in this genus than those

just mentioned. Though it cannot be stated with certainty at present that inversions have played a major rôle in the differentiation of species, the great frequency of this type of structural change points to that fact and makes it quite probable. Considerable chiasma localization, the shortness of the inversions, and the factor of self-sterility have all undoubtedly favored the maintenance of inversions. Together with the possible position effects brought about by these rearrangements, it seems not improbable that inversions have provided the opportunity for genic differentiation to be preserved once it had taken place. However, more data concerning the degree of structural difference between the various species is needed to corroborate the evidence and to extend the information which has been obtained from the hybrid population described in this study, and hybridization between species is being carried out at present to achieve that end.

ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to PROFESSOR KARL SAX for suggestions and criticisms during the course of this study. He is also indebted to DR. A. H. STURTEVANT who, while visiting professor in genetics at this institution, read the manuscript and made several helpful suggestions as to the presentation of the data.

SUMMARY

1. A cytological analysis has been made of two natural hybrids of Tradescantia and of 28 offspring resulting from reciprocal crosses between them. The analysis was concerned with chiasma, inversion bridge, and univalent frequencies, chiasma localization, and pollen sterility. It was found that few irregularities occurred at meiosis in the parental plants, but that a considerable variation was revealed in the hybrid population. The data are presented in table 1.

2. No correlation existed between the frequency terminal chiasmata and the percentage of inversion bridges, but a very high correlation was found between the frequency of interstitial chiasmata and bridge formation. This was interpreted as evidence that interstitial inversions are numerous in this genus, but that generally chiasma localization obscures this fact. With the segregation and subsequent recombination of genetic factors governing meiotic behavior, chiasma localization was interrupted, and crossing over occurred in regions that had previously been more or less isolated, thus revealing the inversions.

3. More than 50 percent of the cells bearing bridges failed to show the usual acentric fragment. This is discussed in some detail.

4. No correlation existed between chiasma frequency and the percentage of cells bearing univalents.

5. Sterility was observed to be always higher than could be accounted for on the basis of meiotic irregularities. Gene unbalance is suggested as a cause of a portion of the sterility, this unbalance possibly resulting from crossovers in included inversions.

6. Experimental data is presented showing that within a certain range, warmer temperatures favor increased chiasma formation particularly in the interstitial regions, with a correlated increase in bridge frequency.

7. Pairing in Tradescantia is initiated at the distal ends of the chromosomes, and proceeds in a proximal direction. Direct and indirect evidence to this effect is given.

8. The control of crossing over is discussed from environmental, structural, and genetical points of view. Genetical factors play a most important part in the determination of chiasma frequency and localization, with the other two factors playing minor rôles.

9. The evolutionary rôle of inversions is discussed. The widespread occurrence of inversions in this genus suggests that these aberrations have played an important part in speciation.

10. Double crossovers in adjacent and overlapping inversions are discussed and diagrammed. Their possible rôle in the production of new chromosomes is brought out.

11. An almost constant ratio exists between the number of interstitial chiasmata per cell and the percentage of bridges when considered for the whole group of offspring. The phylogenetical significance of this ratio is suggested, since in any group of F_2 plants derived from a species cross, it will determine the relative amount of structural divergence between the two species. Its limitations are also discussed.

12. Two factors, opposite in action, are found in Tradescantia. These are (1) self-sterility which facilitates recombination, and (2) inversions together with chiasma localization, which act as barriers to recombination. These barriers are somewhat overcome in the F_2 generation by the segregation and recombination of factors governing meiotic behavior.

13. It is pointed out that self-sterility genes, if located within, or adjacent to, inversions serve as mechanisms for the perpetuation of inversion heterozygosity.

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ON THE NATURE OF X-RAY INDUCED DELETIONS IN TRADESCANTIA CHROMOSOMES

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THAT deficiencies of chromatin account for many of the mutations resulting from X-rays has been well established by the work of DEMEREC, KAUFMANN and SUTTON (1939) and others in Drosophila as well as by that of STADLER (1932) and MCCLINTOCK (1931) in Zea. The production of chromosome aberrations in Tradescantia by the application of X-rays has been thoroughly investigated by SAX (1940). His observations were limited mostly to dicentric and ring types which generally can be interpreted as inviable rearrangements due to gross deficiencies or mechanical difficulties encountered in subsequent mitoses. The relative proportion of viable rearrangements such as inversions and reciprocal translocations could be determined from such data. SAX (1938) has also noted the occurrence of small acentric fragments not involved in other chromosome rearrangements and has proposed that these and small inversions might be attributed to breaks in adjacent gyres induced by a single hit followed by fusion of broken ends into a new association. This theory has also been suggested by BUCK (1939). In view of the fact that such deficiencies may give rise to phenotypic effects and thus appear as mutations, the present investigation on the structure and origin of chromosome deletions was undertaken. A brief abstract of this work has already been published (RICK 1940).

MATERIALS AND METHODS

These studies were limited entirely to one clone of Tradescantia which is free of small centric fragments. This plant is a segregant from a natural cross between *T. canaliculata* Raf. and *T. humilis* Rose. Throughout these experiments inflorescences were subjected to unfiltered X-rays generated by a Coolidge tube with tungsten target operating at 10 ma. at a peak potential of 160 kv. on a line voltage of 115 v. Dosage was always varied by regulating intensity, that is, by regulating the distance from target to inflorescences according to the inverse square law.

Five to ten days subsequent to X-raying, anthers were smeared; preparations were fixed in alcohol-acetic and stained in aceto-carmine. The chromosomes were observed in metaphase and anaphase of the first mitotic division in the microspore.

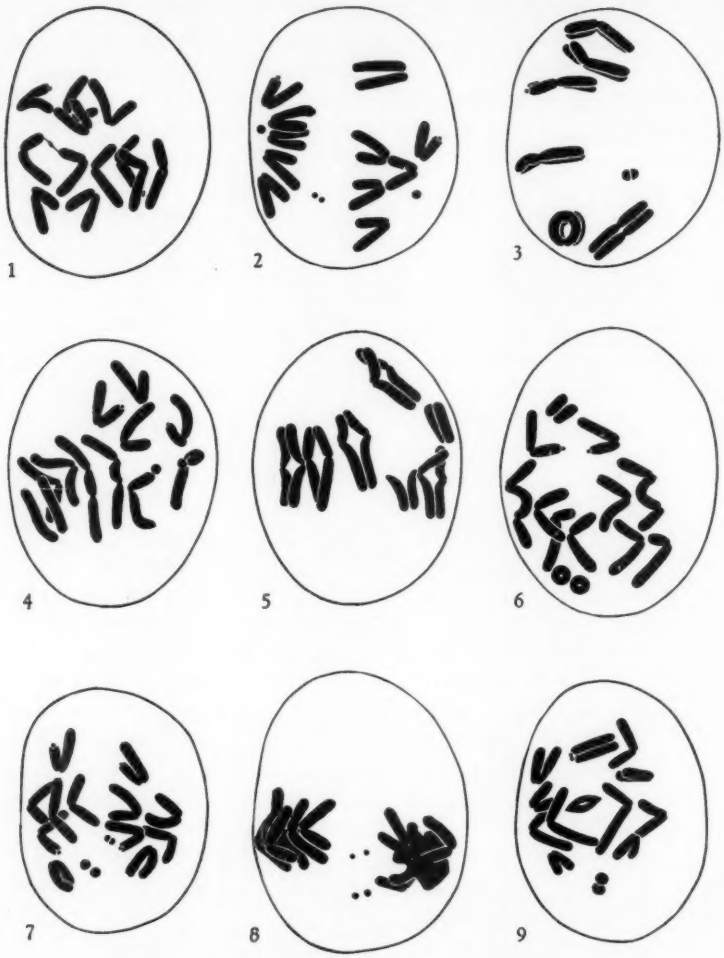
STRUCTURE AND ORIGIN OF DELETIONS

Except for a few rare instances of chromatid aberrations, which can be accounted for as spontaneous breaks (cf. GILES 1940), all rearrangements observed at anaphase and metaphase involved both sister chromatids at the same locus, and thus, as has been well demonstrated by RILEY (1936), MATHER (1937) and SAX and MATHER (1939), are accounted for by breaks induced when the chromosome was effectively single.

As all breaks affect sister chromatids alike, the deletions are always paired and usually in contact, although they may be separated (figures 2, 6, 7, 8 and 9). These deletions are free from other chromosomes excepting rare cases in which they seem to be attached to the ends of separated sister chromatids. Figure 4 exemplifies such terminal attachment, which might conceivably result from unequal interchange, one of the breaks of which occurred in a satellite. At anaphase they show little tendency to separate poleward but remain near the equator or drift to one side, apart from the more orderly centric chromatids.

Regardless of their size, acentric rod fragments bearing two normal chromosome ends are immediately identifiable by their appearance in the same cell with ring or dicentric chromosomes (figures 2, 3, 4, 6, 9). They constitute the exchange terminal deletions of figure 10. Acentric fragments produced independently of such configurations must represent either subtractions from the end of, or portions excised from the interstitial regions of, a chromosome arm, as seen in the simple terminal and simple interstitial deletions respectively of figure 10. Practically all critical evidence has demonstrated that broken ends of chromosomes very seldom remain unfused and the refusion which does occur is always between broken ends (GOODSPEED and UBER 1939). In view of this evidence, the simple terminal deletion should be rare and should appear rod-shaped. Fragments of U-shape, which result from fusion between two broken ends resulting from splitting of terminal deletions have been observed only very rarely. In accord with the above expressed view the simple interstitial deletion would be of ring shape.

The details of internal structure of metaphase and anaphase chromosomes are seldom visible in these preparations. Moreover, small centric ring chromosomes (figure 2) cannot be distinguished morphologically from small rod fragments (figure 3). Thus a morphological distinction between ring and rod fragments can be made only when the length of the chromosome portion deleted considerably exceeds the normal diameter of metaphase chromatids (figures 5 and 6). In a sample of 831 deletions from doses of 200 to 600r, 107 were sufficiently large to be classified definitely into 76 or 9.2 percent of ring shape and 31 or 3.6 percent of rod shape. Of the remaining 724, that is, the great majority of all chromosome deletions



EXPLANATION OF FIGURES 1–9

Camera lucida drawings of microspore division figures from smears prepared 5 to 10 days following X-ray radiation. Magnification about ×1000.

FIGURE 1.—Anaphase showing no aberrations. Both satellites are visible in typical position.

FIGURE 2.—Anaphase showing very small centric ring chromatids and correspondingly large rod (exchange-terminal) fragments in addition to a pair of simple deletions.

FIGURE 3.—Metaphase showing large centric ring chromosome and correspondingly small rod fragment.

FIGURE 4.—Early anaphase showing a pair of simple deletions apparently terminally attached, also dicentric chromatids with rod fragments.

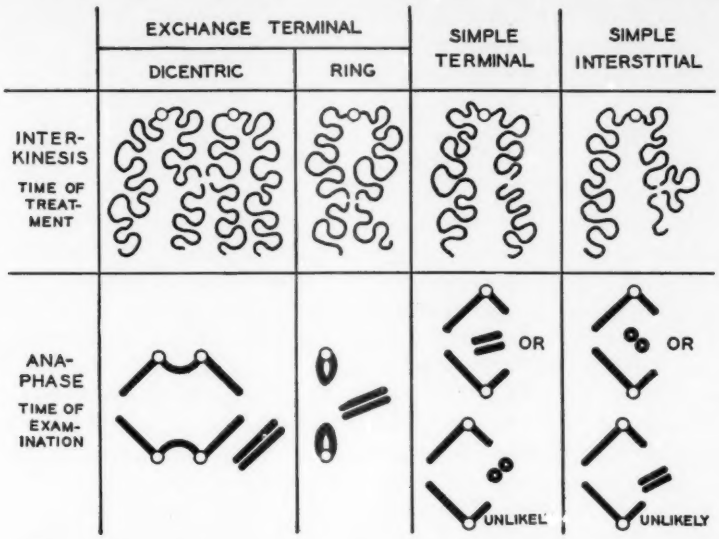


FIGURE 10.—Acentric fragments occurring as chromosome aberrations in Tradescantia.

produced, 71 percent might be expected to be of interstitial origin, if the same proportions occur as in the case of large fragments. Further consideration, however, indicates that this fraction must be much higher.

Where data are available, they indicate (table 1) that, when the frequency of large ring fragments is plotted against X-ray dose, an exponential curve is secured which may be taken as evidence that these are two-hit aberrations of interstitial origin. The designation, "hit," may be regarded as synonymous with ionization because it is the only likely X-ray effect concerned which is produced independently of wave-length. The writer (RICK unpublished) has found breaks in Tradescantia to be of approximately equal frequency at different wave-lengths of applied radiation. A similar finding is reported by EBERHARDT (1939) in Drosophila insofar as a change in phenotypic expression of the dominant allele of *cubitus inter-*

FIGURE 5.—Early anaphase showing a large simple terminal deletion resulting from a break close to the centromere.

FIGURE 6.—Anaphase showing a pair of large ring shaped simple interstitial deletions and deficient arms in addition to dicentric chromatids with fragments.

FIGURE 7.—Anaphase showing two pairs of simple deletions. Four satellites are detectable.

FIGURE 8.—Late anaphase showing two pairs of simple deletions.

FIGURE 9.—Anaphase showing centric ring chromosome, the rod fragment of which bears satellites. A pair of simple deletions is present. Abnormal arm length in chromatids suggests unequal reciprocal translocation.

TABLE 1

Frequency of large ring deletions following three successive doses of X-rays. Time: 8 minutes; smeared 5 days after radiation.

INTENSITY	DOSE	TOTAL CHROMOSOMES	LARGE RING DELETIONS	% RING DELETIONS	EXPECTATION FROM $(D/347)^{1.9}$
25r/min.	200r	5832	22	0.37	0.35
50r/min.	400r	1027	12	1.17	1.29
100r/min.	800r	600	30	5.0	4.7

ruptus is actually a measure of translocation. This is in close agreement with the report by MULLER (1938) that large interstitial deletions in Drosophila vary as the 3/2 power of dosage where radiation was evidently administered at the same intensity but for different lengths of time.

Similar data for large rod deletions are presented in table 2, where the linear relationship between X-ray dose and their frequency argues that they result from one hit and consequently are terminal.

TABLE 2

Frequency of large rod deletions following three successive doses of X-rays. Time: 4 minutes; smeared 5 days after radiation.

INTENSITY	DOSE	TOTAL CHROMOSOMES	LARGE ROD DELETIONS	% ROD* DELETIONS	EXPECTATION FROM $(D/1830)^{1.0}$
25r/min.	100r	5796	4	0.06	0.056
50r/min.	200r	5832	6	0.10	0.117
100r/min.	400r	5220	13	0.25	0.229

* These figures represent a mean of samples (slides) of different sizes and therefore are not exactly equal to the quotient of the two preceding columns.

The nature of the remaining small deletions must be determined by less direct means. In order that a terminal rod fragment may be detected as such, it must involve at least $\frac{2}{3}$ of the average arm of the Tradescantia chromosome. It follows that these result from breaks in the proximal $\frac{2}{3}$ of the arm. SAX and MATHER (1939) have demonstrated that 72 percent of all breaks yielding dicentric chromosomes occur in this region. If the distribution of breaks responsible for terminal deletions is of a similar nature, and there seems to be no reason to doubt this, terminal deletions should constitute only $\frac{31}{0.72 \times 831} \times 100$ or 5.2 percent of all deletions and a still smaller portion of the smaller deletions.

There is no great inequality in length of arm in the chromosome complement of the Tradescantia clone studied here as figures 1–9 will testify; at least calculations based on the assumption that all arms are of equal length should not be far from correct. Two chromosomes in the haploid set bear minute satellites on their shorter arms. Both satellites are detectable in figure 1 and figure 7. In a sample of nine slides from this study it was found that an average of 51.5 percent of the satellites are detectable. For each slide the number of cells in which two, one, and no satellites were observed approximated very well, as judged by the χ^2 test, the values expected from the expansion of $(p+q)^2$ where p = the proportion of all satellites observed for the particular slide and $q = 1 - p$. That there was no excess of cells with only one satellite visible argues that each satellite is equally detectable. This point in addition to the fact that the two arms bearing satellites are of equal length suggests a polyploid origin of these structures (cf. GATES 1939). Now if $\frac{1}{6}$ of the chromosome arms bear satellites and these satellites are detectable in about $\frac{1}{2}$ of the chromosomes, some estimate might be made of the proportion that terminal rod fragments constitute of all the smaller fragments. That they do constitute only a very small fraction is shown by the record of only two satellited fragments in some 1500 small fragments examined. It might be argued that for some reason satellites are less easily detected on rod fragments. Yet as RILEY (1936) points out, acentric fragments show the same developmental cycle as unaltered chromosomes. In the sample of nine slides, the proportion of 200 rod fragments of the exchange terminal type which bore satellites compares very well with values calculated on the assumption that there is equal likelihood of exchange occurring between any two chromosomes (cf. figure 9). Further, this may be taken as evidence that exchanges occur at random between chromosomes.

The relation between the frequency of small simple deletions and dose is given for one series in table 3. Large rod fragments detectable as such are not included here, but large ring fragments are included. Since the latter constitute only about 10 percent of the total, their frequency if different would not seriously affect the result.

In two other series approximating the same number of chromosomes but at different doses, the best fit was secured to curves having as exponents the values 1.80 and 1.62, the average being 1.67. The exponents of exponential curves best fitting exchange ring and dicentric breaks recorded in the same samples were, respectively, 1.94, 2.10, and 1.97, agreeing closely with values obtained by SAX (1939 and 1940). The average exponential curve for simple deletions would be approximated for the dosage range studied here if at the lowest dose 40 per cent were one-hit aberrations and 60 percent two-hit aberrations. Obviously terminal

deletions could not account for this proportion of one-hit deletions. The problem of accounting for this 40 percent will be considered further in the discussion.

Curves like this one representing the relation of a sum of one- and two-hit effects to dosage should approach linearity at very low doses and a parabolic form at much higher doses. Since the dosage range of 100 to 600r units utilized here represents the only part which can be analyzed satisfactorily with the method of scoring used, no test of these trends was possible. Confirmatory results have been published by FABERGÉ (1940) although differences in material, methods of scoring, etc. would not permit a direct comparison. Doses of 1320, 2640, and 3960r secured by varying the intensity, were applied and the method of scoring was merely to record the total number of pieces of chromosomes observed at meta-

TABLE 3

Frequency of small simple deletions following three successive X-ray doses. Time: 4 minutes; smeared 5 days after radiation.

INTENSITY	DOSE	TOTAL CHROMOSOMES	SIMPLE DELETIONS	% SIMPLE* DELETIONS	EXPECTATION FROM $(D/133)^{1.55}$
25r/min.	100r	5796	37	0.64	0.64
50r/min.	200r	5832	117	1.94	1.91
100r/min.	400r	5220	296	5.6	5.6

* These figures represent a mean of samples (slides) of different sizes and therefore are not directly equal to the quotient of the two preceding columns.

phase in the microspore division of *Tradescantia bracteata* Small 70 hours subsequent to X-ray treatment. If breakage and refusion behave at these high doses as they do at lower doses, these pieces should be chromosome aberrations; the only types of these that result in an increased number of chromosome pieces at metaphase are centric ring chromosomes with their rod fragments, and deletions, the latter comprising by far the greater part. Now, if the above interpretation is correct, at the high doses used by FABERGÉ aberrations should increase with the square of applied dosage. If the values obtained are plotted logarithmically with respect to dose and aberrations, the frequency of aberrations is found to vary as the dosage raised to the powers: 2.06, 2.08, 1.55 and 1.38, in four different series, although it is claimed by FABERGÉ that the values obtained do not deviate significantly from those expected if the relation were linear. The discrepancy between the first two and the last two of these values is rather large, but it might not be significant in view of the relatively small number of cells on which these four curves were established.

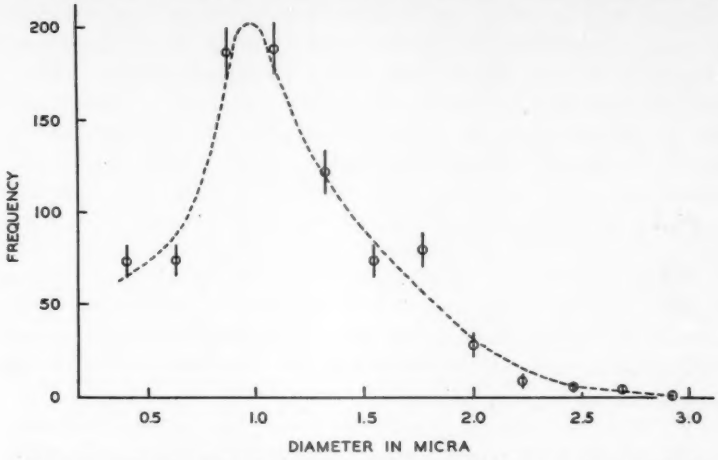


FIGURE 11.—Frequency distribution of deletion size. Vertical lines represent standard deviations of obtained values.

The mean value is 1.77, not much higher than the value obtained above. This result is still probably in keeping with the expected value, 2, for it is known that the division cycle in Tradescantia is notably retarded by heavy X-ray doses, so that even when the natural cycle is at a minimum, some one-hit chromatid aberrations would be expected 70 hours after treatment. These one-hit aberrations are of the simple deletion and single dicentric chromatid plus U-shaped fragment types which would increase the number of chromosome pieces observed.

The values observed by FABERGÉ at these high doses agree remarkably well with values calculated by extrapolation from data in the present experiment, although this might not be expected due to many sources of error such as differences in sensitivity of the material, and possible discrepancies in measurement of ionization which might exist between these experiments. This rough agreement certainly does not prove that the type of aberration at high doses is similar to that at low doses, but it is quite compatible with such an assumption.

Further evidence that simple deletions are chiefly two-hit chromosome aberrations and therefore probably interstitial is to be found in observations of spontaneous breaks. GILES (1940) noted the relative rarity of two-hit chromosome aberrations arising spontaneously and *a fortiori* noted the complete absence of simple deletions in any of his extensive studies.

The distribution of size was recorded for a sample of the deletions studied (figure 11). Since these simple deletions are paired and appear spherical, the diameter was secured by dividing by two the distance across a pair.

Deletions were measured only at metaphase and early anaphase in order to minimize variation due to the coiling cycle. If two breaks occur at random in any chromosome arm where breaks are evenly distributed throughout the arm, the relative frequency of deletions of a given length will be inversely proportional to that length (MULLER 1938). The frequency distribution expected on these grounds may be expressed as a straight line whose equation is $y=(-2N/l^2)\chi+2N/l$ where N = number of fragments and l = length of the arm. Limitation of breaks to any region, as has actually been demonstrated by SAX and MATHER (1939), would tend to increase the expectancy of the smaller deletions, again the increase varying inversely with size of the deletion. Thus, in spite of the fact that the smallest deletions of figure 2 would be most likely to escape observation, the sharp decrease in their numbers in comparison with those clustered around 1μ is probably real.

By similar reasoning, an estimate can be obtained for the proportion of excised deletions that should include the centromere when it is in median position—a reasonably accurate assumption for Tradescantia chromosomes. Under these conditions any deletion larger than half the length of the chromosome will include the centromere, and for smaller deletions the proportion which include the centromere should equal twice the ratio of the length of the deletion to the length of the chromosome. Deletions which include the centromere are, of course, the centric ring chromosomes produced by exchange breaks and those not including the centromere constitute almost all the deletions considered here. If the expected proportion of centric rings for each size is calculated from the data available in this study, a figure of 43 per cent is obtained. The actual proportion of centric rings in this sample is 16 percent.

Another deviation from expected values is to be found in the ratio of deletions to dicentric chromosomes. If breaks are distributed at random to the twelve chromosome arms, and this is supported by evidence encountered above, the probability of two breaks within one arm is 1/12 and that of breaks in two different chromosomes is 10/12, the ratio between the two types of events being 1/10 or 0.10. The proportion of deletions which is produced by two hits, that is, 60 percent at lowest doses, corresponds to the event of two breaks in one arm and the analogue to two breaks in two chromosomes is the dicentric chromosome. The actual ratios between two-hit deletions and dicentrics in three series are 1.20, 1.46, and 2.02, revealing a tremendous excess of deletions above the expected value. CATCHESIDE (1938) and BAUER, DEMEREC and KAUFMANN (1938) reported a similar excess in Drosophila, where the ratio of intra-arm inversions to inter-arm inversions and translocations was $\frac{1}{2}$ compared with the ratio $\frac{1}{4}$ expected on random distribution of breaks.

The reasons for the great discrepancies noted both in the frequency distribution of deletion size and in the ratio of deletions to other types of aberrations are to be sought in the spatial limitation imposed upon refusion of X-ray induced breaks. Other evidence that refusions must be localized and thus influenced by the spatial disposition of the chromosomes at time of treatment is given by BAUER, DEMEREC and KAUFMANN (1938) and SAX (1940). It is well established that relic coils persist through the resting stage and are still detectable at the ensuing prophase and it seems very likely that such coiling can account for these two discrepancies. The existence of relic coils should greatly enhance the opportunity for refusions within a chromosome arm and also for refusions between ends of deletions of certain restricted size classes and these are precisely the two effects noted. The size relation which the fragments of modal frequency in figure 2 bear to the whole chromosome is approximately what would be expected if there are 20–25 minor coils per chromosome as observed in Tradescantia in occasional favorable preparations here and also by SAX and SAX (1935). It should also be noted that during the resting stage considered, any coils which may exist are relic from anaphase chromosomes of the second meiotic division and that numbers of minor coils have been ascertained from anaphase chromosomes of somatic mitoses. Nevertheless, the coiling in both is known to be sufficiently similar to afford this rough comparison.

SAX (1940) has discovered that exchanges between arms of the same chromosome occur much more frequently than expected, if exchanges occur at random between all chromosome arms. Thus, the expectancy of centric rings might be expected to exceed the value of 43 percent calculated above. In spite of this effect, the opportunities for refusion between two breaks within one chromosome arm afforded by relic coiling must be of much greater moment than opportunities for refusion of breaks favored by the proximity of two arms as evidenced by the very low proportion of centric rings obtained.

RELATION OF DELETIONS TO OTHER ABERRATIONS

None of the records suggest a tendency toward either association or dissociation between simple deletions and the exchange breaks, that is, dicentric and ring chromosomes. Where a comparison was made with values expected if these aberrations are produced independently, as in table 4, there is no greater discrepancy than would be expected from random variation.

EFFECT OF TEMPERATURE ON X-RAY INDUCED DELETIONS

The temperature difference was maintained for a period from a few

TABLE 4

Association in the microspore of simple deletions and exchange breaks. 5 slides. 869 microspores. Dose: 400r.

		NUMBER OF SIMPLE DELETIONS							
		NONE 70.6%*		ONE 25.2%		TWO 3.8%		THREE 0.4%	
		OBS.	EXP.	OBS.	EXP.	OBS.	EXP.	OBS.	EXP.
Number of exchange breaks	None** 77.7%	461	478	176	170.5	25	25.6	3	2.6
	One 20.2%	144	124.5	40	44	8	6.5	0	0.7
(Ring and dicentric chromosomes)	Two 2.0%	9	12.2	3	4.3	0	0.3	0	0.1
	Three 0.1%	0	0.6	0	0.2	0	0	0	0

* These percentages are derived from the Poisson distribution of a total of 34.2 percent of simple deletions.

** These percentages are derived from the Poisson distribution of a total of 24.6 percent of exchange breaks.

d.f. = 6 $\chi^2 = 5.656$ $p = 0.3\text{–}0.5$

minutes before radiation to one hour after radiation by warm water and by ice water in cardboard cartons. Hot and cold lots were irradiated simultaneously. Further details of treatment and results are given in table 5.

TABLE 5

Frequency of aberrations induced by X-rays at 3° and 33°C. Intensity: 100r/minute. Time: 3 minutes. Dose: 300r.

TEMP.	RADIATION	TOTAL CHROMOSOMES	DELETIONS*			EXCHANGE BREAKS. RING AND DICENTRIC CHROMOSOMES		
			NO.	%**	RATIO	NO.	%**	RATIO
3°C.	300r	3894	203	5.26	2.85	132	3.37	3.62
33°C.	300r	2988	54	1.84	1	27	0.93	1
3°C.	none	2100	0	0	0	0	0	0

* Deletions include all of the simple type except large rod terminal deletions.

** These figures represent a mean of samples (slides) of different sizes and therefore are not exactly equal to the quotient of the two preceding columns.

Since SAX and ENZMANN (1939) have already demonstrated a much greater yield of two-hit and most of the one-hit aberrations at lower tem-

peratures and since FABERGÉ (1940) noted a similar effect in unclassified aberrations, these results do not seem unusual. Moreover, it is not deemed necessary to experiment further as the former have adequately demonstrated by varying temperature subsequent to radiation that the temperature effect is a secondary one; namely, that the same number of breaks are produced at either temperature, but that refusion is slower at low temperatures, allowing more opportunity for broken ends to move away from old positions and fuse into new associations.

DISCUSSION

From the evidence presented above it is obvious that certain factors promote exchanges of broken ends within the chromosome arm and favor the production of deletions of a certain size class. It also seems very likely that these factors include relic coiling and a spatial limitation to refusion of broken ends. One further point in this connection deserves consideration. SAX (1938) has discussed the consequences of breaks and refusions in adjacent gyres of coiled chromonemata and has indicated that if the coil were fairly compact it is equally likely that a ring fragment deleted in this fashion will encircle or be free of the remaining chromonema. SAX (1940) has also found that in chromatid aberrations fusions resulting in dicentric chromatids are twice as frequent as fusions resulting in reciprocal translocations, indicating that following an effective X-ray hit the broken ends tend to shrink away from the point of the break. Now, if the coiling during the resting stage is relaxed with certain loci in successive gyres closer to each other than others, the shrinkage movement following breaks would favor the formation of free deletions. Actually, no cases of deletions embracing metaphase chromosome arms were encountered. A deletion resulting from breaks in successive gyres would necessarily be small and, when locked around a chromosome arm at metaphase, might be undetectable. At anaphase, however, such a deletion might be expected to interfere with chromatid separation, but such a situation was never observed. On the other hand, deletions so locked might be slipped off the arms of separating chromatids and become terminally attached as in figure 4, yet most deletions are free from chromosome ends. Thus it seems most likely that relic coiling during the resting stage is of a very relaxed and irregular nature, which agrees with the condition insofar as it can be observed directly. It would also follow that small inversions, the formation of which is much favored by tight coiling, would occur relatively much less frequently in this relaxed coiling.

This study affords no criterion whereby one-hit aberrations can be distinguished from two-hit aberrations among the smaller simple deletions resulting from X-ray radiation. The curves obtained, which may be ac-

counted for by 40 percent of one-hit breaks at the lowest dose, include approximately 10 percent of the larger detectable rings which are all two-hit aberrations. Therefore, one-hit aberrations must constitute almost half of the remaining 90 percent of small deletions. Comparisons of size-frequency graphs of fragments produced at different doses show no consistent changes in form except for the greater proportion of larger visibly ring shaped fragments at higher doses, so that the one-hit types cannot comprise any particular size group of the smaller deletions. Might it not be possible that during the resting stage loops within an arm, including relic coils, might bring some portions of the chromosome into closer association than that existing between chromosomes—so close, in fact, that one hit could break both portions? The known spatial limits of the effect of one hit would not discount such an hypothesis, for SAX (1940) has demonstrated that an X-ray hit can break both sister chromatids at the same locus at a time when the split between chromatids can be visibly demonstrated. This point seems to be substantiated also by the work of STADLER and SPRAGUE (1936) who found that X-ray radiation of pollen yields a much lower rate of fractional deficiencies to entire deficiencies than does ultra-violet radiation. A direct cytological comparison of the effects of X-ray and ultra-violet radiation on chromosome aberrations has confirmed these genetic results (SWANSON 1940).

The independent work of several investigators has established beyond doubt the fact that in Drosophila the induced lethal mutation rate behaves as a linear function of the X-ray dosage, and thus the mutation measured is considered to be the result of a single X-ray hit (cf. TIMOFÉEFF-RESSOVSKY 1937). The slight deviation below the linear expectation noted at higher doses is quite close to the proportion of individuals expected to receive two or more mutations if a random distribution is assumed. A similar dosage-mutation rate relation has been revealed in plants where sufficiently investigated (STADLER 1930 and unpublished, GOODSELL 1930). Yet, as SAX (1940) and others have pointed out, if translocations and inversions, which most evidence indicates are the result of two independent hits, account for many of the observed mutations by position effect, then the exponent of the dosage-mutation rate curve should be greater than one. Moreover, if a large proportion of the deficiencies in Drosophila are two-hit aberrations, as found to be the case in the present experiments in Tradescantia, this should be reflected in an exponential trend of the dosage-lethal mutation rate curve.

The situation seems even more paradoxical in view of the finding of DEMEREC, KAUFMANN and SUTTON (1939) that translocations and inversions are directly associated with $\frac{1}{4}$, and deficiencies with $\frac{3}{4}$ of the lethals which display a phenotypic effect in the white-notch region, although

these lethals can scarcely be regarded as a random sample of sex-linked lethals. Further evidence bearing on this problem has been presented by TIMOFÉEFF-RESSOVSKY (1939) who reports that genetically determined inversions, translocations and large deletions which affect the X chromosome increase exponentially with increased X-ray dosage and at the highest dose tested, 6000r, are 40 percent as frequent as X chromosome lethals in the same material. The exact proportion of these chromosome aberrations which exert a lethal effect apparently is not known. Nevertheless, he estimated this proportion to be somewhat less than $\frac{1}{2}$, in consideration of the facts that all deletions measured are lethal in males; that the unbalance caused by segregation in a translocation heterozygote will not affect the measure of X-chromosome lethals; that some inversions and translocations do not produce a lethal position effect; and that some inversions and translocations occurring simultaneously with lethals in the X chromosome are not related in their breakage points to the locus of the lethals. He concluded therefore that the proportion of two-hit aberrations which exert a position effect is so small that even at the dose of 6000 r, this effect on the total lethal mutation rate would be statistically undetectable. This might be the case, if the proportion is very small, yet it scarcely seems possible if the proportion were 10 or 15 percent, which approximates the value given by TIMOFÉEFF-RESSOVSKY. As indicated above, the curve for lethal mutations does not show any such trend, but actually falls below the linear expectation at higher doses in accordance with the expectation of the chance occurrence of two or more mutations in the same chromosome. Certainly, if doses higher than 6000 r could be tested, the mutation rate should reflect the exponential rise of gross chromosome aberrations exerting position effect.

Elimination of gametes bearing two-hit aberrations cannot account for the difference because the method by which BAUER, DEMEREC and KAUFMANN (1938) found the exponential increase in gross aberrations was subject to the same elimination of gametes from treated males as might occur in the detection of lethals by the ClB technique. In view of the considerable sterility known to be induced by X-ray treatment, sterility of F_1 females bearing such aberrations might possibly account for the elimination. Obviously this could not be the case if TIMOFÉEFF-RESSOVSKY (1939) studied F_1 females in the genetic determination of frequency of aberrations. The mating techniques used were not explained in his paper. If this study was based on males, the above suggestion would then be adequate provided that sterility attending aberration is much higher in females than in males.

Since 60 percent of the deletions measured here are two-hit aberrations at the lowest doses and a much higher percentage at the higher doses, it

would follow that the mutations showing a linear relation to X-ray dosage could not be attributed to deficiencies of this sort. The possibility remains that very small one-hit deletions, undetectable due to the limits of resolving power of the microscope, and other minute changes in addition to point mutation are most responsible. This explanation gains support from the citations of MULLER (1938) and MULLER and MACKENZIE (1939) of the unpublished discovery of BELGOVSKY that the smallest deficiencies induced by X-rays in Drosophila vary according to the first power of the dose. Furthermore, DEMEREC (1939) suggested that small deficiencies comprising one to four bands of the salivary gland chromosomes of Drosophila might be one-hit phenomena and that larger deficiencies result from two independent hits because of the great excess of the former above the number expected if breaks were distributed at random. Chromosome deletions in Tradescantia as comparably small as one to four band deficiencies in Drosophila would surely be undetectably small.

DEMEREC (1937) advanced the hypothesis that each effective X-ray hit excises a small portion of the chromosome, and where two such hits occur within certain spatial limits, a refusion between broken ends in a new association would result in larger aberrations in addition to the small deficiencies. However this hypothesis seems to be contradicted by the findings of SOKOLOW (1937) and DEMEREC, KAUFMANN and SUTTON (1939) that few or none of the larger aberrations exhibit deficiencies at their points of breakage. Nor is any other hypothesis plausible which states that the frequency of mutations not associated with gross chromosome aberrations does not bear a linear relationship to dosage. At least in the case of lethals measured by the ClB technique, it seems most likely therefore, as TIMOFÉEFF-RESSOVSKY (1939) has proposed, that the gross chromosome aberrations having a mutational effect are so few in comparison with the total induced mutations that the dosage-frequency relations of the total rate are not statistically affected by the former at the doses ordinarily tested.

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SUMMARY

X-ray radiation of the post meiotic resting nucleus of Tradescantia microspores produces several types of simple deletions in addition to, and independently of, the deletions associated with the formation of ring and dicentric chromosomes. Of the simple deletions induced by X-ray doses of from 200 to 600r, 3.6 percent were visibly rod shaped, 9.2 percent ex-

hibited a detectable ring structure, and the remaining 87.2 percent were too small to be classified. The large rod shaped fragments are probably terminal deletions resulting from single hits (ionizations or ionization clusters) because their frequency is directly proportional to dosage. The large ring shaped deletions are considered to be two-hit aberrations of interstitial origin, since their frequency increases according to the square of the dosage. In the case of the small deletions, considerations of the distribution of breaks in the chromosome arm, and of the proportion of small deletions bearing satellites indicate that they are almost entirely of ring structure and of interstitial origin. The dosage-frequency relations of small deletions may best be accounted for by the hypothesis that at the lowest doses, that is, 100r and 150r, slightly more than one-half are two-hit aberrations and the remainder, one-hit aberrations.

An analysis of the distribution of the sizes of small deletions indicates that they are derived chiefly from breaks in adjacent gyres of the relic coils. Further evidence of the spatial limitation of refusion of broken ends and the consequent effects of relic coils is found in the frequency of small deletions relative to the frequency of ring and dicentric chromosomes. Thus two breaks are necessary for the formation of small deletions, and, as indicated above, these may result from either one or two hits.

X-ray radiation applied at lower temperatures results in a greater yield of chromosome deletions, as also of other aberrations, than does the same radiation at higher temperatures.

All of the chromosome alterations induced by X-rays which result in translocations, large inversions and deficiencies, and most of the visible small deletions in Tradescantia are produced by two independent hits and thus show an exponential increase with increased dosage. The same seems to be the case in Drosophila. Since it is known that comparable chromosome aberrations in Drosophila frequently appear as mutations by virtue of the phenotypic expression of position effects or deficiencies, the dosage-mutation rate curve should reflect this exponential trend according to the proportion of such mutations among all induced mutations. The fact that the dosage-mutation rate curve is strictly linear implies that most of the mutations measured are not the result of gross chromosome aberrations.

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STUDIES OF A TELOCENTRIC CHROMOSOME IN MAIZE WITH REFERENCE TO THE STABILITY OF ITS CENTROMERE

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INTRODUCTION

EVERY chromosome possesses a differentiated region which has been designated by a variety of terms such as 'kinetochore,' 'spindle attachment region,' 'insertion region' and 'centromere.' Beginning with METZNER (1894) it has been believed that this differentiated region, which we shall call the centromere following DARLINGTON, played an essential rôle in chromosome dynamics, being involved in the congression of the chromosomes to the metaphase plate and in their poleward movements at anaphase. Conclusive proof of these probable functions, however, was obtained from the mitotic behavior of acentric fragments which arise through crossing over in heterozygous inversions or by breakage of the chromonemata, and are found to lie as passive bodies, or behave irregularly, upon the acromatic figure.

As is well known it is usually the centromere which leads the way in the anaphase movement of the chromosome towards the pole but there are a few notable exceptions. For example, in the monocentric spindles in Sciara (METZ 1938) one group of chromosomes passes away from the pole even though they show by their orientation, shape, and proximal attenuation that a force located at the centromere is being exerted upon them to move in the direction of the pole. METZ believes that the unusual behavior in these monocentric mitoses indicates the presence of two distinct forces governing chromosome movement. One of these is centered at the centromere while the other appears to be distributed through the length of the chromosome and not centered at one point. However, in the case of bipolar mitoses it is the movement controlled by the centromere which is recognizable and the second force while present and operating cannot be distinguished because of the nature of the bipolar spindle. METZ's observations and BELAR'S (1928) studies with living spindles, in which he found evidence that a portion of the anaphase movement of the chromosomes was due to the expansion of the middle part of the spindle thus pushing the chromosomes apart, make it clear that the centromere is not the sole agent concerned in anaphase movement. That it is an essential one there is no doubt.

* The cost of the accompanying plates has been borne by the Galton & Mendel Memorial Fund.

In recent years a number of investigators, especially SCHRADER (1932, 1936, 1939) and TRANKOWSKY (1930), have become concerned with the structure of the centromere. SCHRADER describes the centromere in the amphibian Amphiuma as a compound body composed of a commissural cup enclosing a minute chromatic spherule which is directly involved in the formation of the half-spindle fiber. The chromatic spherules of SCHRADER are analogous to the kinetic bodies described by SHARP (1934). It may be questioned, however, if the structure of the centromere is identical in all organisms. In maize, where in aceto-carmine smears the centromeres of the paired chromosomes at pachytene have a homogeneous, translucent appearance, the writer has never seen convincing evidence of the presence of chromatic spherules nor were they apparent at meiotic metaphase and anaphase where they should be readily observed (see figure A, Plate 2 and figures A and B, Plate 3). SCHRADER (1939) suggests that the centromeres of Amphiuma and Zea are basically alike in structure and that the apparent dissimilarity between them at meiotic anaphase results from differences in resistance to mitotic separation. However, SCHRADER finds evidence of structural heterogeneity in the centromeres of Amphiuma chromosomes in the late meiotic prophases—a condition which has not been observed in the centric regions of Zea chromosomes, although, it must be admitted, different fixing and staining methods might disclose a more complex structure.

The centric regions of both Zea and Amphiuma exhibit staining reactions which differ from those of the rest of the chromosomes. SCHRADER noted that the centrosomes and the chromatic spherules have similar staining properties and suggested a relationship between these two bodies. POLLISTER (1939) reported a striking correlation between the numbers of centrioles and centromeres in Vivapara where it appears that the centromeres may become disassociated from degenerating chromosomes and take on the properties of centrioles. Unfortunately, in maize the presence of clearly defined centrosomes and chromatic spherules has never been established. Even though the structure of the centric region may not be identical in all organisms, it is apparent that it is a well differentiated region of the chromosome which has a specialized function quite distinct from that of any chromomere.

The centromere is not concerned solely with chromosome movement. It divides the chromosome into two arms which behave in some respects as independent units since the direction of relational coiling in the two arms is at random (SAX 1936) and the genetic phenomenon of interference does not extend from one arm to the other across the centromere. It has also been established, in Drosophila particularly, that the frequency of crossing over is reduced in those regions adjacent to the centromere.

MATHER (1936) has suggested that this reduction in crossing over in the proximal regions may account for their genetical inertness and the accompanying accumulation of heterochromatin. UPCOTT (1937) believes that cell wall formation occurs under centric control. DARLINGTON (1937) believes that the centric region of the chromosome effects the structure of the spindle.

This list of suggested functions of the centromere makes no claim to completeness but it does illustrate that the centromere plays a variety of rôles in mitosis.

The terms telomitic and telocentric have been used to describe chromosomes with an apparently terminal centromere while the terms atelomitic and atelocentric indicate that the centromere is non-terminal. A number of investigators, especially S. NAWASCHIN (1916) and LEWITSKY (1931) who have stated their position clearly and unequivocally, hold that no chromosome has a true terminal centromere. It is maintained that those chromosomes which ostensibly have a single arm possess a minute second arm so small as to escape observation unless special techniques are used at critical stages. That this view may be correct is indicated by the demonstration that a number of rod-shaped chromosomes long believed to be telocentric actually have a sub-terminal and not a terminal centromere. A particularly impressive investigation has recently been made with the minute fourth chromosome of *Drosophila melanogaster* which is so small that it has a dot-like shape in oogonial cells with no indication of being other than telocentric. However, KAUFMANN (1934) from his study of the somatic prophases believed the fourth chromosome to be two-armed. Later, GRIFFEN and STONE (1939) obtained genetic and cytological evidence confirmatory of KAUFMANN'S observation. KAUFMANN also showed that the rod-shaped X chromosome of *melanogaster* has a sub-terminal centromere. The rod-shaped chromosomes of certain Orthoptera have long been held to possess terminal centromeres. DARLINGTON (1936) however states that none of the chromosomes of Chorthippus and Stauroderus possesses a terminal centromere. The joining of two rod-shaped chromosomes at the centromere to form a V-shaped element has been observed in certain Orthoptera (KING and BEAMS 1938). While this has been taken to prove that the rod-shaped chromosomes are telocentric, it is not improbable that they are similar to the X of *Drosophila melanogaster* in that they possess a minute short arm composed of genetically inert material. Unequal translocation could result in the two genetically active long arms becoming attached to a common centromere. Whether or not the two short arms were lost or retained would be of no consequence if they are composed of inert material.

There is apparently no certain case of a telocentric chromosome in the

regular chromosomal complement of any plant, and it is possible, though not as thoroughly established, that a similar condition is true among animals. The failure to find a single undoubted case of a telocentric chromosome in the regular complement of any organism suggests that a centromere so placed is either unable to function properly or is unstable (*cf.* DARLINGTON 1939). The writer (1936) described the occurrence and behavior of a supernumerary telocentric chromosome. It would appear, therefore, that the failure to find terminal centromeres in the normal chromosome complement may be due to their instability and that this instability has led to their disappearance through selection. Further study of the telocentric chromosome mentioned above has yielded data which are pertinent to the consideration of the stability of terminal centromeres.

A TELOCENTRIC CHROMOSOME IN MAIZE

Maize has a haploid set of ten chromosomes. MCCLINTOCK (1933) has shown that each member of the complement can be recognized by its architecture. No member of the regular chromosome complement has a terminal centromere. The fifth longest chromosome has been associated with the *a2-bm-Pr-v2* linkage group. This chromosome has its centromere in a nearly median position. The ratio of the length of the two arms is 1.1:1.0. In certain strains of maize the longer arm often carries a prominent knob which facilitates distinguishing between the two arms. Plants trisomic for chromosome 5 differ markedly in their appearance from disomic sibs. They have thicker, broader leaves with blunter tips, a stubbier tassel, and a shorter stature than do disomes. There is no difficulty in classifying a segregating progeny into disomic and trisomic types.

In 1933 among the progeny of a plant trisomic for chromosome 5 there occurred a single plant which was intermediate in appearance between its trisomic and disomic sibs. A cytological examination of this exceptional plant disclosed that it possessed 21 chromosomes but that the extra chromosome consisted of the short arm only of chromosome 5. It had a terminal centromere. It arose through a break at or in the centromere of a normal chromosome 5. It is difficult to ascertain if the size of the centromere on the short arm of chromosome 5 is identical with that of a normal chromosome 5 because the apparent size of a centromere varies considerably in different cells. The short arm, however, does possess a readily visible centromere approaching in size that of a normal chromosome 5. While there is no proof that this terminal centromere arose from a fracturing of the parental centromere, it is of interest to note that MCCLINTOCK (1932, 1938) has shown that both parts of a transversely broken centromere are capable of functioning.

Previously (1936) this chromosome was described as a 'fragment' chro-

mosome but since this term has been so widely used to denote acentric chromosomes arising either spontaneously or through irradiation the term 'telocentric' is preferred and will be used to describe it. In the 1936 paper the method was described by which the telocentric chromosome consisting of the short arm of chromosome 5 was utilized in placing the genes of the fifth linkage group in the long and short arms of the chromosome. According to the data summarized by EMERSON, BEADLE and FRASER (1935) the order with intervening crossover values of 6 of the 23 genes in this group is *a2* (6) *bm* (6) *bv* (19) *pr* (9) *ys* (32) *v2*. The available linkage data were insufficient to place accurately the remaining 17 genes. Eight of the genes in the fifth linkage group were tested against the telocentric chromosome and the *v2 ys pr v12 v3* and *bt* loci were found to lie in the long arm and *a2* and *bm* in the short arm of chromosome 5. The data presented in table 1

TABLE 1

Summary of Bm Bt Pr backcross data.

F_1 GENOTYPE	PARENTAL COMBINATIONS	REGION 1	REGION 2	REGIONS 1 AND 2	TOTAL
Bm bt pr / *bm Bt Pr*	135 462	8 3	92 268	2 2	972
		1.13%	37.04%	0.41%	
			Bm–*Bt*=1.5%		
			Bt–*Pr*=37.5%		

The inequality of the complementary classes is due to the poor germination of *bt* seed.

show that the order is *bm bt Pr* and that there is only 1.5 percent of recombination between *bm* and *bt* although they lie on opposite sides of the centromere. MCCLINTOCK (1938) from her study of ring-shaped fragments placed *bm* in the short arm close to the centromere. STADLER (1935) obtained a deficiency in the long arm of chromosome 5 which included the *v3* locus but not the neighboring loci of *bm*, *bt* or *bv*. BURNHAM (1934) reported that the order of genes going from the end of the long arm towards the centromere is *v2 ys pr bv bm*. RHOADES (1933b) from a study of a reciprocal translocation indicated that both *bm* and *bt* were close to the centromere of chromosome 5 and BURNHAM reached the same conclusion from his study of another translocation. The above cited data from various investigators give the same placement of loci arrived at by the use of the telocentric chromosome. Utilizing the cytogenetic data it is possible to place both *bt* and *v3* in the linkage map which becomes:

0	6	8	10	12	31	40	72
a2	*bm*	*bt*	*v3*	*bv*	*pr*	*ys*	*v2*

CYTOLOGICAL STUDIES

Synapsis of the telocentric chromosome with the two normal chromosomes 5 was studied in plants hyperploid for the telocentric chromosome. A number of clear pachytene figures was obtained in which the synaptic relationships could be determined (figure 1). At any given region pairing between the three homologous short arms was always in twos, with the

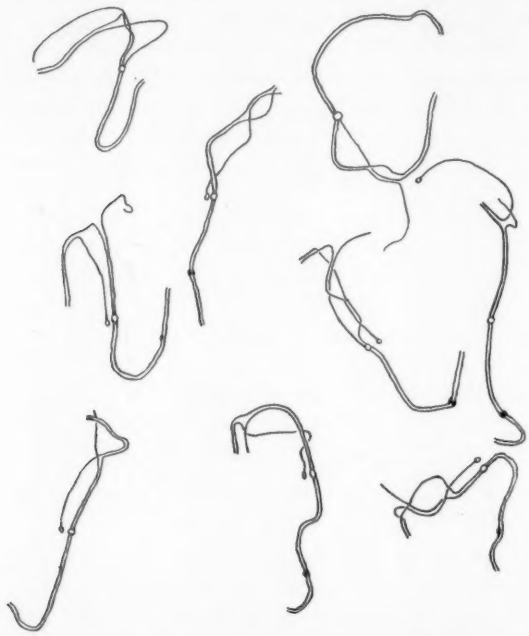

FIGURE 1.—Camera lucida drawings at pachytene showing synapsis of telocentric chromosome with the two normal chromosomes 5. The centromeres are represented by clear ovals or circles and the prominent knob in the long arm, when present, by dark ellipses.

third arm unpaired. Exchanges of pairing mates among the three arms were not frequent; in the majority of pachytene figures there was a single exchange of partners and the greatest number observed was three. It should be clearly understood that the exchange of pairing mates referred to is between the three homologous arms and should not be taken to indicate the existence of chiasmata. It is, of course, true that the observed exchange of partners must occur before chiasmata can be formed between the different chromosomes, but there is no reason to believe that a chiasma is formed in every paired segment lying between the points where exchanges of partners occur, especially if the paired region is short. In fact,

if the partial chiasmatype theory of crossing over is correct, which seems likely, it is not proper to define a chiasma as consisting of an exchange of the pairing elements (that is, chromatids), since sister chromatids are paired on both sides of a chiasma; all that has occurred is a breakage and reunion of ends between two non-sister chromatids. The term exchange of partners is used correctly to describe the exchange of partners among homologous chromosomes in polyploids or in the case of a reciprocal translocation where structural dissimilarity causes a change of mates.

It is evident from figure 1 that pairing between the telocentric chromosome and a normal chromosome 5 does not necessarily commence at the centromere, as the terminal centromere often lies to one side of the two paired centromeres of the normal chromosomes 5; and in some cases was observed 'stuck' to the centromeres of other pairs of chromosomes without preventing synapsis in distally placed regions.

Plate 2, figure B is a photomicrograph of an unpaired telocentric chromosome at pachytene. The equational division or split of this chromosome into chromatids is evident at the distal end. The terminal centromere, which unfortunately cannot be clearly seen in the photograph, appears to be divided or possibly is beginning to divide inasmuch as its distal end is cleft or heart-shaped. As the writer stated in 1936, this apparent division of the terminal centromere at mid-prophase may or may not be representative of the behavior of paired centromeres interstitially located. In a number of organisms the genetic and cytological evidence is convincing that the first meiotic anaphase is reductional for the centric regions of bivalent chromosomes; irrespective of the physical state of division of the centric region of two sister chromatids it acts as a single functional unit. As figure A, Plate 3 suggests, and indeed as has been reported by both SCHRADER and DARLINGTON, each chromatid of the metaphase tetrad may give rise to its own half-spindle component. But SCHRADER points out that it is the chromatic spherules in Amphiuma which give rise to the half-spindle components, and while each chromatid has its own chromatic spherule the commissural cup in which they both lie has not divided so the disjunction of the sister chromatids at the centric region is necessarily reductional. Figure B, Plate 3 also indicates that the bulk of the centric region has not divided even though it clearly shows that each chromatid will form what SCHRADER terms its half-spindle component.

The frequency of trivalent association at metaphase I between the telocentric and the two normal chromosomes 5 was determined in microsporocytes. Metaphase counts were made in five plants and the mean frequency of trivalent association found to be 59 percent. The frequency of trivalents ranged from 50 to 70 percent in different plants. Whether or not this difference is genetic or caused by environmental factors cannot be stated,

but that it is genetic is suggested by the fact that different anthers of one plant gave consistently higher values than did anthers of another. Genetic data to be reported in a later section on the difference between the frequencies of plants hyperploid for the telocentric chromosome in two strains segregating for disomic and hyperploid individuals also argues for a genetic basis.

Figure E, Plate 2 is a photomicrograph of a trivalent group at metaphase I which is typical of the majority of cases where a trivalent occurs. The telocentric chromosome is oriented on the spindle in such a way that it will pass to a pole with one of the normal chromosomes 5, which will disjoin from each other. This non-random orientation of the trivalent, which leads to non-random distribution, is a natural and logical consequence of the equilibrium position attained by the interaction of the three centromeres, which tend to repel each other, and of the chiasmata by which the association of paired chromosomes is maintained through metaphase. In most cells one chiasma, at least, is formed between the two long arms of the two normal chromosomes and one between their two short arms. If in addition a chiasma is formed between the telocentric chromosome and one or other of the two short arms of the normal chromosomes 5, this latter chiasma comes to occupy a terminal position because of the generalized repulsion existing at this time between chromosomes as bodies, in addition to the localized centromere repulsion (*cf.* DARLINGTON 1937). Since both arms of the two normal chromosomes remain associated by chiasmata they will tend to lie symmetrically upon the spindle with their centric regions oriented towards opposite poles. The telocentric chromosome which is associated distally with the normal chromosomes by a triple terminal chiasma will lie more or less in the longitudinal axis of the spindle (that is, at right angles to the equatorial plate) with its terminal centromere directed poleward. The end result is that the centromeres of the two normal chromosomes are oriented against one another while that of the telocentric chromosome is not subject to such regulation. The type of disjunction is not determined by the centromeres themselves. Their orientation on the spindle is a function of the metaphase configuration produced by prophase pairing and chiasma formation. Such metaphase configurations as were commonly observed would be expected to produce anaphase disjunctions in which the two normal chromosomes 5 disjoin from each other with the telocentric chromosome accompanying one or other of the normals. These configurations rarely should give an anaphase distribution in which the two normals pass to the same pole while the telocentric chromosome goes to the opposite one. The genetic data in tables 2–5 amply confirm this expectation.

When the telocentric chromosome is a member of a trivalent group it passes poleward at the same time at which the bivalents are disjoining. It is recognizable during anaphase I because, possessing a terminal centromere, it has a V-shaped appearance resulting from the attachment of the two chromatids at the undivided centromere while their distal ends are some distance apart. The other dyads in anaphase I have a double V- or double J-shaped appearance, depending upon the relative lengths of their two arms, with the apices of the V's or J's attached to a common centromere.

In prophase II the two monads of the telocentric chromosome, which will separate equationally in the subsequent anaphase, form a single rod-shaped chromosome as they are conjoined by the still undivided centromere. The repulsion between the two chromatids is so pronounced at this stage that they tend to lie in a straight line. The centromere does not appear in its usual position but is forced to one side and the two chromatids appear fused at their proximal ends. This specious appearance is even more clearly illustrated in the case of the other dyads, especially those where the two arms differ greatly in length. Here the two short arms appear joined into a single element while the two long arms also appear united into a single body, the two elements being separated by the centromeric region (figure G, Plate 3). When the equational separation of the two chromatids of the telocentric dyad occurs in anaphase II, the monads (chromatids) appear as rod-shaped bodies with the terminal centromere leading the way to the pole. The other monads appear as single V's or J's, depending upon the location of the centromere (figure J, Plate 2).

The unpaired telocentric chromosome usually lay on the spindle at metaphase I but in some cells failed to move onto the plate (figure G, Plate 2). Its behavior at anaphase was variable. After the bivalents had completed their anaphase movements, its two halves often would separate equationally and the daughter univalents would begin their migrations to opposite poles. That they were able occasionally at least to complete their journey to the poles in time to be included in the interphase nuclei was made evident by the observation of daughter univalents in prophase II. The equational separation of the univalent occurs so much later than the disjunction of the bivalents that the writer previously (1936) believed they failed to divide equationally in the first meiotic division, but this was an erroneous conclusion drawn from the study of too early anaphases. While the univalent usually divided equationally in anaphase I, it did not always do so because it was not uncommon to find the chromosomes at the two poles in interphase with the univalent lying to one side in the cytoplasm. Presumably it was those univalents that congressed which

later divided equationally at anaphase while those that failed to congress become laggards. When they lie near a pole, lagging univalents may in some cases be drawn fortuitously into the telophase nucleus.

When a daughter univalent succeeded in reaching the pole in anaphase I, it lagged in the following anaphase because it had precociously undergone the equational separation which normally occurs in the second division (figures H, I, Plate 2). No detailed counts were made but it seemed that the number of lagging chromosomes at anaphase II was less than twice the number of univalents which split equationally in the first division. UPCOTT (1937) noticed a similar phenomenon in Tulipa and suggested that some of the daughter univalents were carried to the poles in anaphase II along with the dividing chromosomes. Another possibility is that the daughter univalents which do not reach the poles in anaphase I fail to congress at metaphase II and, lying off of the spindle, would not appear to be lagging. This point is worthy of more study, but the cytological observations indicate that the telocentric chromosome was almost invariably lost through lagging at the first or second meiotic divisions when it was unpaired at metaphase I. The genetic data on the frequency of hyperploid individuals in the progeny of a hyperploid plant is the best evidence that the unpaired telocentric chromosome usually suffered elimination. Conversely, the telocentric chromosome underwent normal meiotic behavior when it was a member of a trivalent group. In brief, the cytological observations show that (1) the usual orientation of the trivalent group on the metaphase plate was of such a nature as to lead to a nonrandom distribution in anaphase. The gametes should consist mainly of two types, namely those that are haploid and those hyperploid for the telocentric chromosome, with relatively few gametes having two normal chromosomes 5 or the telocentric chromosome only. (2) The observed frequency of trivalents at metaphase permits the prediction of the relative numbers and types of offspring expected in the progeny of a plant hyperploid for the telocentric chromosome, since the unpaired telocentric is usually eliminated when it is an univalent.

GENETIC STUDIES WITH THE TELOCENTRIC CHROMOSOME

The *a2* and *bm* loci were reported by the writer (1936) to lie in the short arm of chromosome 5. A strain was obtained hyperploid for the telocentric chromosome carrying the recessive *bm* allele in each of the normal chromosomes 5 and the dominant allele in the telocentric chromosome. These hyperploid plants were crossed reciprocally with diploid *bm* individuals and the progeny classified for the *bm* character and the different chromosomal types. Table 2 summarizes the results obtained using the hyperploid individuals both as the male and female parent in backcrosses. The data

show that 98.91 percent of the progeny obtained when hyperploid plants were used as the male are diploids homozygous for the recessive allele *bm*. These arose from the functioning of a haploid pollen grain carrying *bm* in a normal chromosome 5. Eighteen of the 7,245 plants or 0.25 percent were diploids not exhibiting the *bm* character and therefore carried the dominant allele in the chromosome contributed by the hyperploid parent. This type of chromosome arose from a crossover between the telocentric chromosome and a normal chromosome in the *bm*-centromere interval so that the dominant allele was transferred to the normal chromosome. Thirty-seven or 0.51 percent of the offspring were hyperploid for the telocentric chromosome and were identical in constitution with the male parent. These individuals arose through the functioning of a pollen grain carrying the telocentric chromosome with the dominant allele and a normal chromosome 5 with a recessive allele. As the cytological observations show that something over thirty percent of the grains should be hyperploid for the telocentric chromosome, the genetic data show that the hyperploid grains do not successfully compete with haploid pollen and that it is only an occasional hyperploid grain which effects fertilization. Hyperploid pollen grains are well filled with starch and cannot be distinguished in appearance from haploid grains. The ineffectiveness of hyperploid grains in accomplishing fertilization in competition with haploid pollen is probably due to a slower rate of pollen tube growth.

Two *bm* individuals were primary trisomes of chromosome 5. These plants arose from a gamete with two normal chromosomes 5. If they were contributed by the male it follows that two events must have occurred. First, the disjunction in anaphase I must have been such that the two normal chromosomes 5 went to the same pole. That such disjunction occurs is shown by the data in the same table where 1.63 percent of the eggs received two normal chromosomes 5. Second, such a hyperploid pollen grain with two normal chromosomes 5 must have functioned in competition with haploid pollen. That such grains occasionally do compete successfully was shown in the experiment in which related primary trisomes of chromosome 5 were used as the male parent, five plants in the total of 1,212 offspring being primary trisomes. The product of the frequency of the two events gives a probability of less than one such individual expected where two were observed. There is also the possibility that an egg with two chromosomes 5 arose in the diploid female parent through non-disjunction. Such spontaneous occurrences of primary trisomes have been observed but they are so rarely found that nothing is known of their frequency. It seems not unreasonable to suppose that the two primary trisomes arose from a male gamete in the manner suggested.

The foregoing classes with their observed frequencies can be readily ac-

counted for. In addition to them, however, there is a class which is entirely unexpected as it includes a type of chromosome absent in either parent. This class consists of the 22 secondary trisomes which possess a supernumerary chromosome consisting of two short arms of chromosome 5 attached to a single, median centromere. Twenty-one of the secondary trisomes were *Bm* and one was *bm*. This suggested that the telocentric chromosome was involved in the formation of the secondary or iso-chromosome since it carried the *Bm* allele. The exceptional *bm* secondary trisome

FIGURE 3.—The seedling to the left is a secondary trisome in which the secondary chromosome is composed of two short arms of chromosome 5 attached to a median centromere. The secondary chromosome arose through misdivision of the centromere of a telocentric chromosome consisting of the short arm of chromosome 5. The seedling to the right is a sibling of the secondary trisome and is a diploid. The differences in growth and texture of the leaves are quite pronounced between the secondary trisome and its disomic sib.

could be accounted for by a crossover between the telocentric chromosome and a normal chromosome 5. It also is possible that it arose from a normal chromosome 5 by transverse division of the centromere. Data presented in other tables leave no doubt that the telocentric chromosome is involved in the formation of the new type of chromosome. The secondary trisomes were strikingly different in appearance from any of the other chromosomal types. Their dwarf-like habit and thick leaves of leathery texture made them easily recognizable as seedlings (see figure 3) while the other chromosomal types could not be accurately classified until a much later stage.

Hyperploid plants used as female parents in backcrosses with diploid *bm* individuals yielded progenies distinctly different in some respects from those obtained when the hyperploid plants were used as the male parent. As table 2 shows, there were 10 diploid *Bm bm* plants out of a total of 5,523, or a percentage of 0.18. This value agrees very well with the percentage of 0.25 for the same class when the hyperploids were used as the male. These plants originate as before from a crossover between the telocentric chromosome and a normal chromosome 5 in the *bm*-centromere region. There were 3,738 (67.68 percent) diploid *bm* individuals. This class comes from haploid gametes with non-crossover chromosomes. The frequency of this class is much lower than in the reciprocal backcrosses as there were 1,671 or 30.26 percent hyperploid plants while only 0.51 percent of the offspring were hyperploid *Bm* plants when hyperploid individuals were used as the male parent. The difference between the two kinds of backcrosses in the frequency of the hyperploid *Bm* is due to the fact that there is little or no competition between megaspores. If the basal megaspore of the quartet happens to receive the telocentric chromosome in addition to a normal chromosome 5, it develops without competition into a functional embryo sac; but a hyperploid pollen grain never enjoys such a positional advantage and must compete against haploid spores. The data obtained when hyperploid plants were used as female parents also differ from the reciprocal cross in that five hyperploid individuals with the recessive *bm* allele in all three chromosomes were found in the former while none were observed in the latter data. These five hyperploid *bm* plants arose from a crossover which transferred *bm* from a normal chromosome to the telocentric chromosome which later passed to the same pole as a *bm*-bearing normal chromosome 5. Similar gametes arising in microsporogenesis would fail to survive because of their relative inability to function in competition with haploid spores. Likewise 1.63 percent of the plants were primary trisomes homozygous for *bm* in progenies obtained when hyperploid plants were used as female parents while only 0.03 percent were found in similar progenies when the hyperploid plants were used as the male parents. There is no reason to believe that the actual frequency of such gametes is greatly different in the two sexes; it is simply a matter of the presence or absence of competition from haploid spores. The number of *bm* primary trisomes obtained when hyperploids were used as female parents is a measure of the frequency with which the two normal chromosomes 5 go to one pole while the telocentric chromosome passes to the other. Likewise the number of individuals hyperploid for the telocentric chromosome is a measure of the frequency with which one of the normal chromosomes passes to the same pole as the telocentric chromosome. The data show that there were 90 of the former to 1,671 of the latter type

of disjunction. This is precisely the type of behavior predicted from the cytological study of pairing and orientation on the spindle at metaphase I. As in the crosses where the hyperploid plants were used as the male parent, there occurred the unexpected class of secondary trisomes. Nine secondary trisomes were found; all of them were *Bm* which indicates that the telocentric chromosome was involved in the formation of the secondary chromosome.

The *bm* locus lies in the short arm of chromosome 5 close to the centromere. Distal to *bm* lies the *a2* allele some 10–12 crossover units removed. Data from plants carrying the dominant allele of the *a2* locus in the telocentric chromosome and recessive alleles in the two normal chromosomes 5 should parallel those reported for *bm* except that the greater crossover distance of *a2* from the centromere should alter the relative frequency of certain classes of offspring. These data are given in table 3. They agree very well with those for *bm*. The percentage of diploids with the dominant allele is 1.74 and 0.82 when hyperploids were used as the male and female parents, respectively. The *A2*-bearing normal chromosome arises through crossing over in the *A2*-centromere interval. The higher frequency of diploid *A2 a2* offspring obtained when hyperploids were used as the male parent in backcrosses suggests a higher crossover value in male than in female flowers. This is in agreement with unpublished data of the writer's which show for chromosome 5 significantly higher crossover values in male flowers as compared with the female. In both types of backcrosses the percentage of *A2 a2* individuals is several times greater than the percentage of *Bm bm* plants. This follows from the fact that *a2* is further removed than *bm* from the centromere.

In the data obtained when hyperploids were used as male parents, the percentage of hyperploid plants with the telocentric chromosome carrying *A2* is 0.42. In addition there were 2 (0.04 percent) individuals hyperploid for a telocentric chromosome with *a2*. These arose through crossing over followed by the functioning of a hyperploid grain and would consequently be found rarely. The total percentage of individuals hyperploid for the telocentric chromosome is 0.46 which is similar to the percentage of 0.51 in table 2 for the *bm* data. One primary trisome homozygous for *a2* was found. It could have arisen spontaneously in the diploid female parent or from the functioning of a pollen grain with two normal chromosomes 5 each with *a2*. As in the case of those hyperploid plants used in the *bm* experiments these also throw the unexpected class of secondary trisomes. There were 26 secondary trisomes in a total population of 5,450 or a percentage of 0.48 when hyperploid individuals were used as male parents. All 26 secondaries were *A2* in appearance which supports the conclusion

drawn from the *bm* data that the telocentric chromosome is involved in the formation of the secondary chromosome.

In the offspring of hyperploids used as the female parent 1,411 in a total of 5,605 individuals, a percentage of 25.17, were hyperploid for the telocentric chromosome. Thirteen hundred and eighty-six of them were phenotypically *A2* and 25 had *a2* in the telocentric as well as the normal chromosomes 5. Since the *A2* locus affects aleurone color an attempt was made to determine the genotypic constitution of all 1,386 plants hyperploid for the telocentric chromosome and carrying an *A2* allele by pollinating them with *a2* pollen. Successful pollinations were made on 1,159 plants. In all but one plant the aleurone ratios indicated the presence of a single *A2* allele borne by the telocentric chromosome. The single exception had *A2* in both the telocentric and a normal chromosome 5. This exceptional plant is a consequence of a crossover in the *A2*-centromere region between the telocentric and a normal chromosome, following which the two chromosomes involved in the crossing over passed to the same pole at anaphase I. One of the four possible combinations formed at the end of the second division would have an *A2* allele in both the telocentric and the normal chromosome 5. The 25 *a2* plants hyperploid for the telocentric chromosome also arose from crossing over in the *A2*-centromere region. If, following a crossover between the telocentric and one of the two normal chromosomes in this region, the assortment of the telocentric chromosome is at random with respect to the two chromosomes 5, there will result 3 $\frac{a}{a}$ to 1 $\frac{A}{A}$ combinations at the end of the second division. If the two crossover chromosomes always disjoin to opposite poles the expected ratio is 2:0 while a ratio of 1:1 is expected if they always pass to the same pole. The observed ratio of 25:1 indicates that the two crossover chromosomes usually pass to different poles. This correlation between crossing over and disjunction is similar to that found in triploid Drosophila. It should be mentioned that of the 1,385 individuals listed in table 3 as having their single *A2* allele in the telocentric chromosome only 1,158 were actually proven by genetic tests to be so constituted. However, since only one exceptional individual was found in the total of 1,159 tested, little error is introduced by this classification.

The percentage of *a2* plants hyperploid for the telocentric chromosome is 0.45 and the percentage of *bm* plants of similar chromosomal constitution, from table 2, is 0.09. This difference can be ascribed to the relative positions of the two loci with respect to the centromere. There were 48 primary trisomes homozygous for *a2*. This percentage of 0.86 is approximately

half the percentage of *bm* primary trisomes from a similar type of cross. The total frequency of individuals hyperploid for the telocentric chromosome in the *a2* data is 25.17, which is lower than the percentage of 30.35 found in the *bm* data. The reduced percentages of both primary trisomes and plants hyperploid for the telocentric chromosome in the *a2* data compared to the *bm* data suggest a lower frequency of pairing of the telocentric chromosome with the normal chromosomes 5 in the *a2* strain than in the *bm* strain. It is likely that this difference is genetically conditioned but nothing is known of its basis.

There were eight secondary trisomes in the progenies obtained using hyperploids as the female parent. All eight possessed the *A2* allele which again indicates that the telocentric chromosome was involved in the genesis of the secondary chromosome.

Data obtained from hyperploid plants possessing the dominant allele in the telocentric chromosome and the recessive allele in each of the normal chromosomes are the most illuminating since the dominant allele serves as a marker for the telocentric chromosome. However, in addition to these data a number of progenies were obtained from hyperploid plants which had a dominant allele in one of the two normal as well as in the telocentric chromosome. Data from a single combination of this type are presented in table 4. They will not be discussed in detail as they confirm in all respects the conclusions reached from the data in tables 2 and 3. The chief point of interest in these data is that secondary trisomes arise with a low but consistent frequency whenever the telocentric chromosome is present.

The data presented on the inheritance and behavior of the telocentric chromosome have been derived from individuals in which a single locus, either *a2* or *bm*, was followed in the telocentric chromosome. Data were obtained, in addition, from hyperploid plants in which both the *a2* and *bm* loci were marked by mutant alleles and the two long arms of the normal chromosomes were carrying the *Pr* and *pr* alleles.

The dominant allele at the *a2* locus produces aleurone color in the presence of *A*, *C* and *R* while *a2* results in colorless aleurone. In the hyperploid plants used these three complementary genes were homozygous dominant and only the *a2* locus was heterozygous, so a classification for *A2* and *a2* could be made before planting. The *Pr* and *pr* gene pair determines whether the color is to be purple or red, *Pr* conditioning purple and *pr* red color. In *a2* seeds it is impossible to classify for the *Pr pr* pair. The *a2* locus is also concerned in plant color so a check on the aleurone classification into colored and colorless was possible.

The constitution of the hyperploid plants for the three loci was *A2 Bm /A2 bm Pr/a2 bm pr;* the composition of the telocentric chromosome being listed first. These hyperploid plants were pollinated by triple recessive

pollen and the resultant seed divided into purple, red, and colorless classes. The ensuing progenies from the three classes of seed were classified for the *bm* character and for the various chromosomal types. These data are given in table 5. As a consequence of *Pr* lying in the same chromosome with *A2* while *pr* is in the *a2* bearing chromosome, the purple and red seed produced different percentages of the various classes. Among the progeny from *Pr* seed there were 471 diploid *bm* plants and only 199 from *pr* seed. The *A2 bm Pr* plants arose from non-crossover chromosomes (crossovers between the *A2 bm Pr* chromosome and the telocentric chromosome in the *A2 bm* region could not be detected) while the *A2 bm pr* individuals arose from crossovers between *pr* and *A2*. Equal numbers of plants hyperploid for the fragment should be found in the *Pr* and *pr* classes if the telocentric chromosome shows no preference with which normal chromosome 5 it disjoins in anaphase I. There were 281 and 350 hyperploid plants in the *Pr* and *pr* classes, respectively, which indicates no pronounced preferential assortment although the deviation of 35 from equality is somewhat suggestive. Another striking difference between the *Pr* and *pr* progenies is that there were 50 primary trisomes homozygous for *bm* among the *Pr* individuals while only four were found in the *pr* class. Those in the *Pr* class can be simply accounted for by non-disjunction of the two normal chromosomes. The four primaries homozygous for both *pr* and *bm* likewise arose from non-disjunction of the two normal chromosomes, but the fact that they came from gametes with two chromosomes 5 each with the recessive *pr* gene which was present in but one of the parental chromosomes indicates that a crossover in the *pr*-centromere interval took place between the chromatids of the two normal chromosomes followed by their non-disjunction in the first meiotic division. Since anaphase II is equational for the centromere, one-fourth of the combinations should carry two *pr* chromosomes while three-fourths should be *Pr Pr* and *Pr pr* in the ratio of 1:2 respectively. Approximately 12 of the 50 primary trisomes in the *Pr* class arose, therefore, in such a manner. There were two secondary trisomes present in both the *Pr* and *pr* classes. Of the 675 plants from *a2* seed all but two were diploids homozygous for *bm*. The two exceptions were hyperploid plants with a telocentric chromosome carrying *Bm*. These arose from a crossover in the *A2-Bm* region between the telocentric chromosome and the *a2-bm-pr* chromosome followed by their non-disjunction in anaphase I. One of the four possible combinations formed at anaphase 2 would possess a telocentric chromosome with the *a2 Bm* alleles and a normal chromosome of *a2 bm* constitution.

The genetic data presented in tables 2 to 5 inclusive are of interest in two respects. First, since it was possible to recognize all of the various chromosomal types arising as products of the meiotic process, extensive

genetic data were obtained which afforded a check on the observed cytological behavior of the telocentric and the two normal chromosomes in meiosis. Second, and of more interest since it is pertinent to the question of the stability of the terminal centromere, the data show that the telocentric chromosome was regularly involved in the genesis of a new chromosome equivalent to two short arms of chromosome 5 with a single, median centromere. Since adequate data have been presented on the frequency with which this new chromosome type arises, we will next consider the manner in which it originates.

ORIGIN OF SECONDARY CHROMOSOME

When plants hyperploid for the telocentric chromosome were used as the male parent a grand total of 19,242 offspring was obtained of which 86, or 0.45 percent, were secondary trisomes. Of a total of 17,175 offspring obtained when the hyperploid plants were used as the female parent there were 27, or 0.16 percent, secondary trisomes. This comparison is unfair since approximately 30 percent of the offspring in the latter crosses consist of hyperploids similar to the female parent, while less than one-half of one percent of such individuals were found when the hyperploids were used as the male parent. If the frequency of secondaries is calculated from the data obtained with the hyperploids as the female parent (omitting the hyperploid class from the total) the percentage of secondaries is 0.22. This value is about half that obtained when the hyperploid was the male parent. The relative frequencies of secondaries in the direct and reciprocal backcross data are in striking contrast to those of the other hyperploid types, namely the classes hyperploid for a telocentric chromosome or a whole chromosome 5. Although approximately 30 percent of the pollen grains possessed a supernumerary telocentric chromosome, only 0.46 percent of the offspring were hyperploid for this chromosome. It is certain that the hyperploid grains are at a great disadvantage against haploid pollen and only rarely succeed in functioning. Data have also been presented which show that grains hyperploid for a normal chromosome 5 are rarely functional in competition with haploid grains. It is likewise certain that there is little or no competition between euploid and aneuploid megaspores since the frequencies with which the different chromosomal types appear in the progenies obtained when the hyperploid is the female parent are reasonably close to those expected on the basis of pairing and disjunction at the first meiotic division in the microsporocytes. We are then faced with an anomalous situation in the frequency with which secondary trisomes appear when plants hyperploid for the telocentric chromosome are used as the pollen parent. There is no reason to believe that the frequency with which the secondary chromosome arises is enough

higher in the male flowers to account for the relatively high number of secondaries transmitted through the pollen, especially in view of the fact that pollen hyperploid for either the telocentric chromosome or chromosome 5 rarely functions even when present in large numbers. The possibility that two extra short arms of chromosome 5 have no detrimental effect on the pollen, while a single extra short arm is highly deleterious, and consequently that grains hyperploid for this secondary chromosome are as capable of functioning as haploid grains seems most unlikely. It is rendered untenable by the following experiment.

The secondary trisomes proved to be highly sterile in both the male and female flowers. Although the anthers have few aborted grains they are rarely extruded from the glumes and consequently shed no pollen. If, however, the mature anthers are removed and the pollen manually extracted, small quantities of viable grains can be obtained. When this pollen was applied to diploid silks a number of seeds were obtained. A total of 623 plants were grown from such seed and all proved to be diploids. It follows that those pollen grains hyperploid for the secondary chromosome were not able to function against haploid pollen. That they were present was proved by a study of the chromosomal complement of microspores at the first microspore division. Their frequency was not ascertained, however, because it was not always possible to differentiate between a supernumerary secondary chromosome and an extra normal chromosome 5. However, approximately 10 percent of the female progeny of a secondary trisome consist of secondary trisomes, so it is not unreasonable to assume that a like percentage, at least, of the pollen grains were hyperploid for the secondary chromosome. The failure to find a single secondary trisome in the offspring of a secondary used as the male parent makes it reasonably certain that the secondary chromosome so upsets the normal balance that the hyperploid grains are unable to successfully compete with haploid grains.

Before suggesting two possible mechanisms whereby the secondary chromosome may be transmitted through the pollen, it may be pertinent to consider the development of the male gametophyte which has been studied by a number of investigators. Essentially the story is as follows: The nucleus of the microspore divides to form a generative and a vegetative or tube nucleus. The generative nucleus divides again to form the two sperm cells. The mature male gametophyte or pollen grain at the time of anthesis contains three haploid nuclei—the vegetative nucleus, which is in a metabolic condition, and the two sperm nuclei. When the pollen grain germinates a pollen tube is extruded through the germ pore, enters the silk and grows down the silk towards the ovule. The vegetative nucleus assumes a position near the growing tip of the pollen tube and it is be-

lieved that the growth of the tube is under its control. It has been assumed that the two sperm are passive bodies playing no effective role in the activities of the pollen tube, merely being transported down the silk to the embryo sac.

The transmission of the secondary chromosome through the pollen may be readily accounted for if it is assumed that in a microspore with a telocentric chromosome the sequence of events is as follows: In the first microspore mitosis the telocentric chromosome splits equationally into two chromatids. Normally at anaphase each of these two chromatids possesses its own centromere and they pass to opposite poles. Rarely, however, the terminal centromere of the telocentric chromosome either fails to divide or divides transversely so that the two chromatids find themselves attached at their proximal ends to a common centromere. At anaphase this newly constituted chromosome with a median centromere and two identical arms passes to either the vegetative or generative pole. In the event that it moves to the generative pole, the end of the first microspore division finds a haploid vegetative nucleus and a generative nucleus hyperploid for the secondary chromosome. The two sperm formed by the division of the generative nucleus will each carry the secondary chromosome. A pollen grain of this constitution presumably would not be under any handicap during its stylar journey because it possesses a haploid vegetative nucleus. It carries, however, a sperm which will give rise to a secondary trisome if it fertilizes a haploid egg.

The postulated mis-division of the centromere of the telocentric chromosome has never been observed by the writer at anaphase of the first microspore division. DARLINGTON (1940), however, states that he observed the formation of iso-chromosomes in microspores of Fritillaria resulting from the delayed division of newly arisen telocentric chromosomes. An attempt was made to observe the phenomenon cytologically but the low frequency of its occurrence (about 9 in a 1,000 judging from the number of secondaries in the offspring) and the difficulty of identifying individual chromosomes at the microspore anaphase proved to be insuperable difficulties. While it was not possible to observe the genesis of the secondary chromosomes in the manner postulated the evidence at hand suggests that this mechanism or a similar one gives rise to the secondary chromosome.

In addition to the above hypothesis there is another possible way in which the telocentric chromosome might give rise to the secondary chromosome. KOLLER (1938), UPCOTT (1937) and especially DARLINGTON (1939) have shown that the centromere of an univalent chromosome sometimes divides transversely at meiosis in such a way that the two short arms are joined together and the two long arms are also attached to one piece of centromere. That is, the misdivision of the centromere gives rise to two

isochromosomes. In the case of the telocentric chromosome it has been observed that it is often unpaired by meiosis and that it sometimes fails to split equationally in anaphase I. It is possible, though it has not been cytologically demonstrated, and indeed it would be difficult to do so with certainty with a telocentric chromsome, that the centromere of an occasional telocentric chromosome divides transversely in either the first or second meiotic divisions to form an isochromosome with two identical arms. This newly formed isochromosome fails to reach either pole and forms a micronucleus. It must be further assumed that it persists until the microspore division where through its fortuitous position in the cell it is occasionally incorporated into the telophase group at the generative pole. The end result here is the same as in the first hypothesis, namely that the vegetative nucleus is haploid while the generative nucleus is hyperploid for the iso- or secondary chromosome. This hypothesis has the advantage that the misdivision of the centromere is postulated to occur in the meiotic divisions, where univalents of other plants have been observed to misdivide, and not in the somatic division of the microspore. While it has been necessary to invent the two hypotheses primarily to account for the transmission of the secondary chromosome through the pollen, it is highly probable that whatever mechanism is responsible for the origin of the secondary chromosome in the male flowers is also responsible for its origin in the female flowers.

The secondary trisomes originating from plants hyperploid for the telocentric chromosome have been studied cytologically. Figures C, D, E and F, Plate 3 and figures A, B and C, Plate 1 show that the secondary chromosome is composed of two short arms of chromosome 5 with a median centromere. A study of synapsis reveals that the order of loci in the secondary chromosome is *a b c d e* centromere *e d c b a* which is the order expected from the postulated mechanisms. When the secondary chromosome is a univalent but forms a chiasma between its two homologous arms a ring of one is found at diakinesis (figure C, Plate 3). The associations of the secondary and the two chromosomes 5 at pachytene shown in figures A, B and C, Plate 1 are explicable only if the 'secondary' chromosome consists of duplicate arms. The cytological behavior of these secondary trisomes is similar to that of the secondary for the short arm of chromosome 5 which arose spontaneously (RHOADES 1933a) in a stock disomic for chromosome 5. The genetic data disclose that the telocentric chromosome is involved in the formation of the secondary chromosome since with one exception (which can be accounted for by a crossover) the same alleles are present in the two chromosomes. (No secondary trisomes were found in the thousands of offspring from disomic sister plants.) These data also indicate that pollen hyperploid for one or two short arms of chromosome

5 rarely functions, yet the frequency of secondaries transmitted through the pollen is certainly no less than through the eggs. All of the above enumerated facts point to the correctness of the hypothesis that the secondary chromosome arises through mis-division of the centromere of the telocentric chromosome and as a consequence of this mis-division the generative and vegetative nuclei of the male gametophyte differ in their constitution.

Irrespective of the precise manner in which the secondary chromosome arises, it is a reasonable inference that its formation results from the instability of the terminal centromere of the telocentric chromosome. Whether its misbehavior consists of failure to divide or of a transverse instead of a longitudinal division cannot be stated with certainty but the observations of KOLLER (1938), UPCOTT (1937) and DARLINGTON (1939) on the transverse centromere division of univalent chromosomes at meiosis make the latter probability more likely. Evidence has been presented which suggests that in the formation of the secondary chromosome the mis-division of the terminal centromere occurs during or immediately following meiosis. An experiment was undertaken to determine if the telocentric chromosome was unstable in sporophytic mitoses. When plants hyperploid for the telocentric chromosome with the *Bm* allele, the two normal chromosomes 5 carrying *bm*, are used as female parents approximately 30 percent of the offspring are hyperploid for the telocentric chromosome. These individuals are *Bm* phenotypically because the telocentric chromosome bears the dominant allele. If, however, the telocentric chromosome or that part carrying the *Bm* locus is lost during the development of the sporophyte the recessive brown mid-rib character is expressed in the deficient portions of the plant. Three hundred hyperploid individuals were closely examined for the presence of *bm* sectors, and 22 or 7.3 percent were found possessing them. In these 22 plants the *Bm* allele present in the telocentric chromosome had been eliminated during development of the sporophyte. In several of the plants the deficient sectors extended into the tassel and a cytological examination was made of microsporocytes lacking the *Bm* allele. In one case the telocentric chromosome had been completely eliminated while in four other plants the telocentric chromosome had become diminished in size. In two of these instances there was a small fragment with a subterminal centromere; a second plant had a chromosome with a terminal centromere but only half the length of the parental telocentric chromosome; the third had an extremely small chromosome fragment consisting of nothing more than a terminal centromere with two or three chromomeres.

In the few cases studied cytologically no indication was found that the *bm* variegation was due to a reciprocal translocation occurring in a somatic

cell and resulting in somatic segregation such as JONES (1938) reports for the endosperm of maize.

A similar experiment was conducted in which the telocentric chromosome was marked with the dominant *A2* allele while the normal chromosomes 5 carried the recessive allele. The *B* and *Pl* alleles were also present in this stock so the *A2 B Pl* plants had a purple plant color. If *A2* was lost during the development of the sporophyte the tissue deficient for this allele would be brown instead of purple. Five hundred and one purple hyperploid plants were examined at maturity for brown sectors and 31 or 6.2 percent possessed them. The *a2* sectors must have arisen through the loss of *A2* in telocentric chromosome. The size of both *bm* and *a2* sectors varied from small to large. No cytological study has as yet been made of the *A2* losses. It appears, however, from the study of certain of the *Bm* losses that an unchanged telocentric chromosome was present in the early embryo and that some alteration occurred during development because the non-deficient cells possessed a complete telocentric chromosome while the deficient cells had a reduced or missing telocentric chromosome. If elimination of the telocentric chromosome in somatic tissue sometimes occurs through the transverse division of the centromere in a manner similar to that believed to happen at meiosis, the sectorial plants should show an asymmetry produced by the marked differences in appearance and texture between diploid and secondary tissues. In no variegated plant was tissue characteristic of the secondary found. Secondary chromosomes may arise in somatic cells but no evidence that they do has been obtained. The simple explanation of the transverse division of the centromere will not account for the origin of the diminutive chromosomes. It must be admitted that the nature of these structural changes is unknown but that they are a consequence of the terminal position of the centromere can be argued with some reasonableness. The secondary chromosome is a direct product of the telocentric chromosome but has a median rather than a terminal centromere. If the instability of the telocentric chromosome is due to some factor other than the unusual position of its centromere it would be expected that the secondary chromosome would also be unstable. However, nearly 200 secondary trisomes have been obtained during the course of these studies and no evidence of instability of the secondary chromosome, exhibited either as asymmetrical sectors of growth or variegation, has been found. It is the writer's experience, and he understands also of other maize students, that sectors due to loss or somatic segregation are rarely found in the sporophyte. This is true of trisomic as well as disomic plants. Apparently a chromosome with an interstitial centromere is more stable than one with a terminal centromere.

THE STRUCTURE OF THE CENTROMERE

The centromere appears at pachytene in maize chromosomes stained with aceto-carmine as a simple body with a homogeneous, translucent appearance. There is no suggestion of the compound nature which SCHRADER found for the centromeric region of Amphiuma. In Amphiuma the centromere is a compound body composed of the commissural cup and spindle spherule. The spindle spherule is connected with the half spindle component. Presumably this is a function reserved for the spherule and in case of its loss the commissural region would be unable to form a half spindle fiber. It would seem that the centromeric region of a maize chromosome lacks this specialization of its component parts because MCCLINTOCK (1932, 1938) found that both parts of a fractured centromere were able to function in a normal manner. NEBEL (1939) believes that the centromere is a compound body consisting of three parts: a central achromatic body, the chromatic kinetic bodies (equivalent to spindle spherules), and the chromatic connecting chromomeres of the chromonemata with the achromatic body. If the centromere is broken he assumes that the kinetic body will be regenerated by that part of the achromatic body not retaining it after breakage. In the case of the maize contromere it is difficult to determine the formation or loss of an invisible body. It seems probable that in maize there is no differentiation of the centromeric region into recognizable structures having specialized duties but that on the other hand any part of the centromere region, providing it is not attached to an inordinately large piece of chromatin, is able to function normally. In this connection it should be noted that MCCLINTOCK found that each part of a fractured nucleolar-organizer body was able to function.

DARLINGTON (1939) from a consideration of the transverse division of the centromere of unpaired chromosomes reached certain conclusions concerning its internal structure. He concluded that it possesses a dual nature consisting of a fluid and a fibrous element. The fibrous elements or centrogenes normally control the plane of division or 'explosion' of the fluid element. Since the fibrous elements lie across the centromere the fluid will usually divide in the plane of division of the centrogenes. DARLINGTON accounts for the observed misdivision of the centromeres of univalent chromosomes by assuming that the centrogenes apparently divide after the chromonemata and misdivision is due to their failing (exceptionally) to divide in time for the explosion of the centric fluid which is precocious in univalents at meiosis. DARLINGTON'S conclusions regarding the internal structure of the centromere are admittedly speculative, but it seems to the writer that he is justified in assuming some internal organization within the centromere to account for its normally orderly longitudinal division. Whether or not his conception of the cause of misdivision of the centro-

mere is correct there is no doubt that misdivision does occur since it has been observed cytologically by DARLINGTON, KOLLER, and UPCOTT in addition to the evidence presented in this paper on the genesis of the secondary chromosome.

In considering the ways by which the secondary chromosome could have arisen from the telocentric chromosome, it was suggested that a terminal centromere might be 'sticky' and would occasionally become attached to another terminal centromere especially if they were in close proximity for a considerable time. That is, if two telocentric chromosomes were in the same cell their centromeres might fuse to form a metacentric chromosome. Following RANDOLPH'S (1932) technique, root tips of seedlings hyperploid for the telocentric chromosome were submerged in hot water to induce doubling of the chromosomes. After treatment the root tips were fixed and sectioned. A dozen clear polar views at metaphase of cells with the double number of chromosomes were found and in each the two telocentric chromosomes although lying parallel to one another were clearly separate. Though the heat treatment produced a restitution nucleus with double the number of chromosomes, the terminal centromeres of the two telocentric chromosomes did not fuse after lying in juxtaposition for some hours. These observations are of such a fragmentary nature as to merit little weight but they indicate that the formation of the secondary chromosome occurs when the centromere of the telocentric chromosome has misdivided and not from 'unsaturation' of terminal centromeres.

LEVAN (1938) believes that the division of the centromere is delayed by the alkaloid colchicine. When root tips were treated with colchicine he found at metaphase what he describes as c-pairs formed by the attachment of the two daughter chromosomes to their undivided centromere. The inactivation of the spindle apparatus produced by colchicine is believed to be connected with a delay in the division of the centromere. (This is in agreement with SCHRADER'S and DARLINGTON'S conception of the centromere as playing an important rôle in the development of the spindle.) After a time the centromere divides and the two daughter chromosomes come to lie free from each other but in parallel alignment. Since DARLINGTON believes that the misdivision of the centromere of unpaired chromosomes at meiosis is due to the failure of the centrogenes to divide in time for the explosion of the centric fluid, it seemed possible that a delayed division of the centromere of the telocentric chromosome produced by colchicine treatment might invariably result in the formation of the secondary chromosome. Healthy root tips of plants hyperploid for the telocentric chromosome were submerged in an 0.2 percent aqueous solution of colchicine for one hour. Twenty-four hours later the material was fixed and sectioned. A number of clear figures were found in which doubling had oc-

curred but in no case were the two telocentric chromosomes attached at the centromeric region. More extended observations of both colchicine and heat treated material might have shown an occasional secondary chromosome but there is reason to doubt if either treatment would be effective. Misdivision of the centromere of univalent chromosomes occurs either at or immediately after the meiotic divisions. The centromere of a univalent is, however, at this time in a pecularíar situation compared to the paired centromeres of a bivalent and its misdivision results from an aberrant precocious attempt to divide one mitosis in advance of the usual time. In the colchicine and heat-treated material all of the chromosomes are subject to the same forces concomitantly and there is no more delay in the division of the centromere of the telocentric chromosome than of the other centromeres of the chrosome complement.

ON THE ORIGIN OF SECONDARY TRISOMES

Secondary trisomes were first reported by BELLING and BLAKESLEE (1924) in Datura. Since each chromosome is two-armed and the secondary chromosome consists of two homologous arms incorporated into a single chromosome there are two possible secondary trisomes for each chromosome. In Datura all 12 of the possible primary types have been found but only 14 of the 24 secondaries have been discovered (BLAKESLEE and AVERY 1938). In a number of other plants including maize and *Nicotiana sylvestris* all or nearly all of the primary types have been isolated. Secondaries have been rarely reported. The writer (1933a) described a secondary for chromosome 5 in maize and one has been reported by PHILP and HUSKINS (1931) in Matthiola. Recently, GOODSPEED and AVERY (1939) believed they had found several secondary trisomes in *Nicotiana sylvestris* but their classification was based on the appearance of the plants and not on cytological examination so final judgment must be withheld concerning the true nature of their supposed secondaries.

BELLING and BLAKESLEE (1924) suggested that the secondaries originated from a reversed synapsis of two homologous chromosomes and that a crossover occurred at the only place where homologous parts were together which would be the centromere. This hypothesis can be rejected as improbable. BLAKESLEE and AVERY (1938) suggest "that unequal crossing over between parallel sister strands in such a way as to retain spindle attachment points for each newly organized chromosome which has been formed by joining together by their broken ends the two similar halves of the strands affected" might account for the origin of the secondaries. However, the results reported here and by the writer in 1938 as well as the cytological observations by KOLLER, UPCOTT and DARLINGTON make it probable that the secondaries arise through transverse division of the

centromere. While only in the maize secondary has it been established that the secondary chromosome is a strict isochromosome it seems probable that the others are also of a similar structure and have all arisen through misdivision of the centromere. Secondaries might arise directly from an unpaired chromosome by misdivision of its centromere as has been observed cytologically by the investigators mentioned above, or from misdivision of a telocentric chromosome as reported in this paper. The low frequency with which secondary trisomes arise from the telocentric chromosome makes it likely that in many instances the secondaries arise directly from the misdivision of unpaired atelocentric chromosomes although a telocentric chromosome is a potential source of secondaries. If, in organisms with no telocentric chromosomes in the normal complement, secondaries come only from telocentric chromosomes, their frequency would be the product of the probability of a telocentric fragment arising and the probability that once having arisen it would be transformed into a secondary chromosome. There are no data available from which the correlation of the frequency with which telocentric chromosomes arise through misdivision or other causes and the frequency of secondary trisomes can be determined. Since both telocentric chromosomes and isochromosomes were observed by KOLLER, UPCOTT and DARLINGTON as products of the misdivision of the centromere, it is not likely that all secondaries come progressively from telocentric chromosomes followed by misdivision of the centromere. Judging from the data reported in this paper for the maize telocentric chromosome the misdivision of its centromere is a relatively rare event. However, in considering the origin of secondary or isochromosomes it is of interest to note that recently DARLINGTON (1940) followed the behavior of telocentric chromosomes arising through misdivision of the centromere at meiosis in the first microspore division. He states that "Following misdivision of the centromere at meiosis in diploid and triploid Fritillaria new telocentric chromosomes are formed whose broken ends rejoin within the centromere. This type of chromosome is delayed at metaphase and anaphase in the pollen grain mitosis. It may then either break again at the centromere or pass without separation to the pole as a new isochromosome." It is not known whether or not this delayed division of the Fritillaria telocentrics in the pollen grain division also exists in the sporophytic divisions of the following generation. In the case of the maize telocentric reported in this paper there is good reason to believe that the type of misdivision occurring in the gametophyte division does not happen in the somatic division of the sporophyte.

The data compiled by BLAKESLEE and AVERY (1938) on the frequency of secondaries from diploids and related primaries are in agreement with DARLINGTON's thesis that misdivision of the centromere of univalent chro-

mosomes gives rise to secondaries or isochromosomes. In a trisomic plant one of the three homologous chromosomes is often unpaired which is precisely the condition favoring misdivision. Actually, the Datura workers found that the secondaries were thrown by related primaries 14 times as frequently as by diploids.

The secondary chromosome formed by the misdivision of the telocentric chromosome has two identical arms which is true of the attached X's in *Drosophila melanogaster*. It is doubtful, however, if the attached X's arose by a misdivision of the centromere, although such an origin is a possibility, since L. V. MORGAN (1938) has shown that two X chromosomes may become attached by replacement through crossing over of the two arms of a Y chromosome by X's.

HÅKANSSON (1932) reported a chromosome in Triticum with like ends. While he believes it arose through crossing over in a duplicated segment and its two arms therefore not wholly equivalent, it is possible that it is a true isochromosome and arose through misdivision of the centromere. LOVE (1939) reported ring univalents in Triticum which may be isochromosomes. HUSKINS and SPIER (1934) and LOVE (1938) have reported a chromosome in Triticum with a terminal centromere due to the loss of one arm. It is of some interest to know if these telocentric chromosomes will give rise to isochromosomes. BLAKESLEE and AVERY (1938) state that a telocentric chromosome in Datura consists of the ·11 part of the 11·12 chromosome. If this chromosome has a truly terminal centromere it should form an occasional 11·11 secondary.

RANDOLPH (1928a) has described a type of supernumerary chromosome in maize known as the B-type. MCCLINTOCK (1933) found that the centromeres of the B-types were terminal although DARLINGTON (1937) believes them to be sub-terminal. DARLINGTON'S conclusions were drawn from a study of somatic metaphase plates where he observed a constriction near one end which he interpreted to be the centric constriction. MCCLINTOCK, however, studied the pachytene stage where a much clearer picture of the morphology is obtainable and her published photograph of two paired B-types shows a terminal centromere. While it is possible that there are different kinds of B-types and that those which DARLINGTON studied possessed sub-terminal centromeres, it is not unreasonable to suppose that the constriction observed by DARLINGTON marks the junction of the euchromatin and the deeply staining pycnotic bodies (heterochromatin?) so clearly seen in the pachytene chromosome. While there is some dispute concerning the location of the centromere of the B-type chromosome, if we accept MCCLINTOCK'S findings, as the writer does, it is of some interest that RANDOLPH (1928b and unpublished) has found a series of diminutive chromosomes. All of them probably descended by fragmentation from an

original B-type since LONGLEY (1938) found that a diminutive chromosome frequently synapsed with B-types. This behavior of the B-type corresponds to the fragmentation of the telocentric chromosome reported in this paper and it is not improbable that in both instances the unstability is due to the terminal location of the centromere.

The data reported in this paper on the behavior of the telocentric chromosome leave no doubt that this chromosome is unstable. It gives rise to an isochromosome through misdivision of its centromere and it was also found to undergo structural changes in somatic divisions leading to loss or diminution in size. The mechanism of the latter changes is unknown but the greater frequency of their occurrence in the telocentric chromosome makes it probable that they are a result of the terminal position of the centromere.

S. NAWASCHIN in 1916 declared that no chromosome in the normal complement of any organism possessed a terminal centromere. This is true for plants and may hold for animals. If the behavior of all terminal centromeres is similar to the one reported in this paper the absence of telocentric chromosomes is understandable because the unstability of terminal centromeres would lead to the elimination of chromosomes possessing them.

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During the course of the four years in which the data reported here were obtained it was necessary to determine cytologically the chromosome constitution of literally hundreds of plants in order to insure that the visual classification of the various chromosome types were accurate. Without the efficient help of VIRGINIA H. RHOADES this would have been too arduous a task to have been accomplished and the writer wishes to express his appreciation of her invaluable assistance.

SUMMARY

Maize plants hyperploid for a telocentric chromosome consisting of the short arm of chromosome 5 produce an occasional secondary trisome. The supernumerary chromosome of the secondary trisomes consists of two short arms of chromosome 5 attached to a median centromere. It was shown through the use of mutant genes lying in the telocentric chromosome that it was involved in the formation of the secondary chromosome. The frequency with which secondary trisomes were found when plants hyperploid for the telocentric chromosome were used as the pollen parents was 0.46 percent, while their frequency was only 0.22 percent when the same plants were used as female parents. Pollen hyperploid for either one or two short arms of chromosome 5 rarely functions successfully in competition with haploid grains. It is suggested, therefore, that the secondary chromo-

some arises at meiosis from a transverse division of the centromere of the telocentric chromosome, and that it is occasionally incorporated into the generative nucleus during the first microspore division. The vegetative nucleus would be haploid and pollen tube growth normal but the two sperm would transmit the secondary chromosome.

Data have been obtained which indicate that the telocentric chromosome undergoes structural changes in somatic cells. The production of secondary or isochromosomes at meiosis from the telocentric chromosome and its loss and modification in somatic tissue show that a terminal centromere is unstable. Such a telocentric chromosome would tend to be eliminated by natural selection. This instability may apply to all telocentric chromosomes and account for the fact that telocentric chromosomes are rarely, if ever, found in the normal chromosome complement of any organism.

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TABLE 2

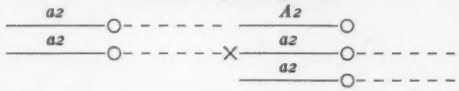
Summary of the data on progenies obtained using plants hyperploid for the telocentric chromosome as male and as female parents.

Type of cross: *bm* / *bm* ○- - - - - - × *Bm* / *bm* / *bm* ○ *and reciprocal*

CHROMOSOMAL CONSTITUTION	OFFSPRING OBTAINED WHEN THE HYPERPLOID PLANTS WERE USED AS THE PARENTS INDICATED			
	MALE PARENT		FEMALE PARENT	
	NUMBER	PERCENT	NUMBER	PERCENT
Bm / *bm* ○- - - - - - / *bm* ○- - - - - -	18	0.25	10	0.18
bm / *bm* ○- - - - - - / *bm* ○- - - - - -	7166	98.91	3738	67.68
Bm ○ / *bm* / *bm* ○- - - - - - / *bm* ○- - - - - -	37	0.51	1671	30.26
bm ○ / *bm* / *bm* ○- - - - - - / *bm* ○- - - - - -	0	0.00	5	0.09
Bm ○- - - - - - / *bm* ○- - - - - - / *bm* ○- - - - - -	0	0.00	0	0.00
bm ○- - - - - - / *bm* ○- - - - - - / *bm* ○- - - - - -	2	0.03	90	1.63
Bm ○ *Bm* / *bm* ○- - - - - - / *bm* ○- - - - - -	21	0.29	9	0.16
bm ○ *bm* / *bm* ○- - - - - - / *bm* ○- - - - - -	1	0.01	0	0.00
	7245	100.00	5523	100.00

TABLE 3

Summary of the data on progenies obtained using plants hyperploid for the telocentric chromosome as male and as female parents.

Type of cross:  *and reciprocal*

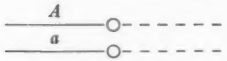
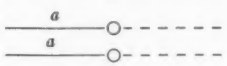
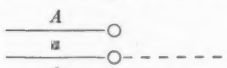
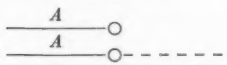
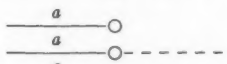
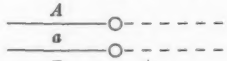
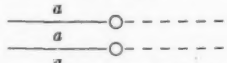
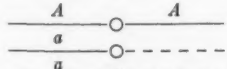
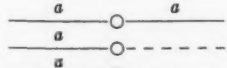
CHROMOSOMAL CONSTITUTION	OFFSPRING OBTAINED WHEN THE HYPERPLOID PLANTS WERE USED AS THE PARENTS INDICATED			
	MALE PARENT		FEMALE PARENT	
	NUMBER	PERCENT	NUMBER	PERCENT
	95	1.74	46	0.82
	5303	97.30	4092	73.00
	23	0.42	1385	24.71
	0	0.00	1	0.02
	2	0.04	25	0.45
	0	0.00	0	0.00
	1	0.02	48	0.86
	26	0.48	8	0.14
	0	0.00	0	0.00
	5450	*100.00*	5605	*100.00*

TABLE 4

Summary of data when $\frac{Bm}{Bm}$ $\frac{Bm}{bm}$ *individuals were used as the male in backcrosses to diploid bm plants. The phenotypes of the different chromosomal classes are in the second column.*

CHROMOSOMAL CONSTITUTION	PHENOTYPE	OFFSPRING OBTAINED	
		NUMBER	PERCENT
——○- - - - - - ——○- - - - - -	*Bm*	2596	48.06
——○- - - - - - ——○- - - - - -	*bm*	2755	51.00
——○ ——○- - - - - - ——○- - - - - -	*Bm*	22	0.40
——○ ——○- - - - - - ——○- - - - - -	*bm*	0	0.00
——○- - - - - - ——○- - - - - - ——○- - - - - -	*Bm*	0	0.00
——○- - - - - - ——○- - - - - - ——○- - - - - -	*bm*	0	0.00
——○—— ——○- - - - - - ——○- - - - - -	*Bm*	29	0.54
——○—— ——○- - - - - - ——○- - - - - -	*bm*	0	0.00
		5402	*100.000*

TABLE 5

Summary of data when $\frac{A2\ Bm}{A2\ bm}$—○ $\frac{Pr}{pr}$ *individuals were used as the female in backcrosses to diploid a2 bm pr plants.*

CHROMOSOMAL CONSTITUTION	PHENOTYPE	NUMBER OF OFFSPRING
	a bm	673
	a Bm	0
——○- - - - - -	*A Bm Pr*	2
——○- - - - - -	*A Bm pr*	1
	A bm Pr	471
	A bm pr	199
	a bm	0
	a Bm	2
——○	*A Bm Pr*	278
——○- - - - - -	*A Bm pr*	350
——○- - - - - -	*A bm Pr*	3
	A bm pr	0
	a bm	0
	a Bm	0
——○- - - - - -	*A Bm Pr*	0
——○- - - - - -	*A Bm pr*	0
——○- - - - - -	*A bm Pr*	50
	A bm pr	4
	a bm	0
	a Bm	0
——○——	*A Bm Pr*	2
——○- - - - - -	*A Bm pr*	2
——○- - - - - -	*A bm Pr*	0
	A bm pr	0

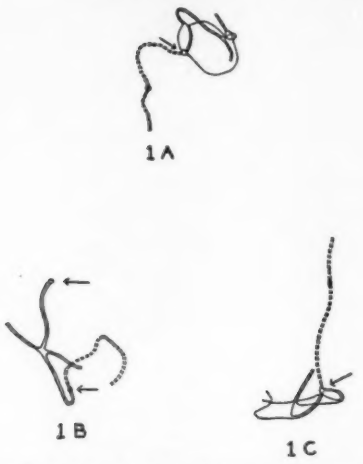
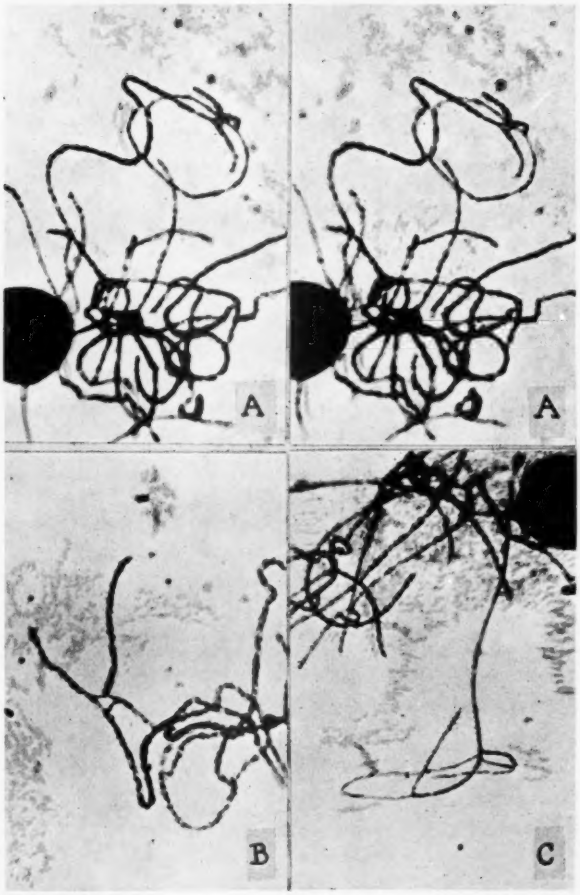
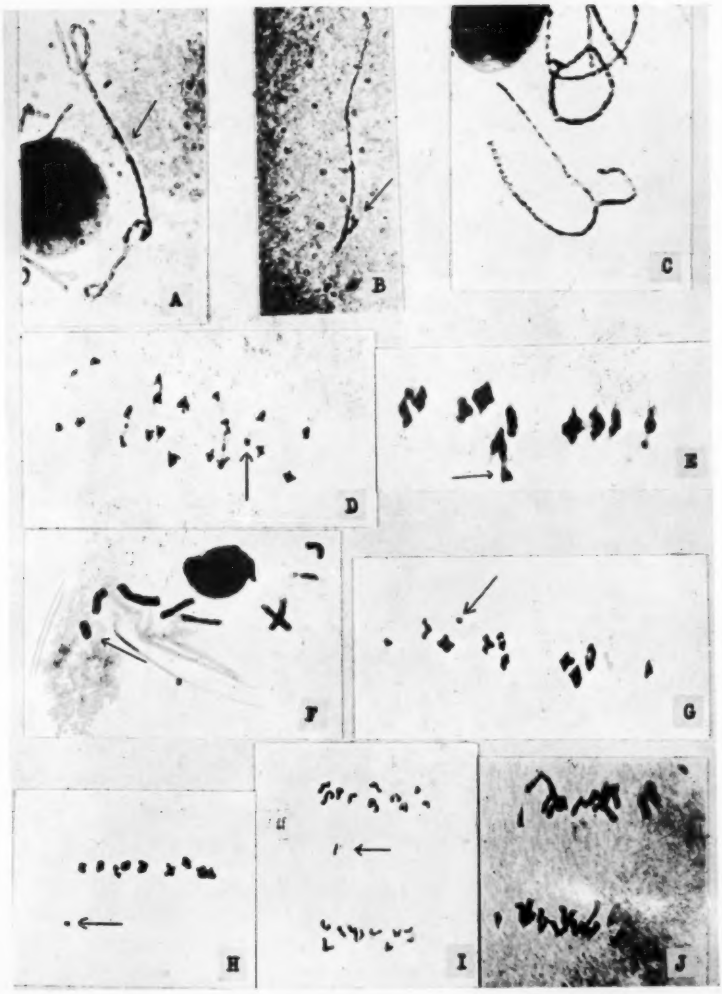


FIGURE 2.—Diagrammatic sketches of the pachytene configurations shown in Plate 1. The centromeres are represented by bulges and are indicated by arrows. The long arms of chromosome 5 are shown by broken lines while the short arms are indicated by solid lines. Figure 1 A corresponds to figure A, Plate 1, figure 1 B to figure B, and figure 1 C to figure C in Plate 1.

EXPLANATION OF PLATE 1

Figures A, B and C show synapsis at pachytene between the secondary chromosomes and the two normal chromosomes 5. The two photomicrographs of figure A are at different levels. Figure B is from a secondary trisome that arose in a stock disomic for chromosome 5 (RHOADES 1933a). The more intimate pairing seen in figure B is not a characteristic difference between this secondary trisome and those arising from the telocentric chromosome. See figure 2 for interpretation.


A
A
B
C


A
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D
E
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G
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I
J

EXPLANATION OF PLATE 2

FIGURE A.—Photomicrograph of two chromosomes 5 paired at pachytene. The centromere is indicated by arrow.

FIGURE B.—Photomicrograph at pachytene of unpaired telocentric chromosome. The equational split is evident at the distal end. The terminal centromere is indicated by the arrow and was clearly terminal.

FIGURE C.—Photomicrograph at pachytene of telocentric chromosome with its centromere stuck to the centromeres of two paired chromosomes 10. The distal end of the telocentric has a foldback, that is, it is paired hon-homologously in this figure. There is no suggestion that the telocentric chromosome is two-armed.

FIGURE D.—Early anaphase showing disjunction of the paired homologues while the unpaired telocentric chromosome is still on the plate. In late anaphase it may separate equationally and its two chromatids (daughter univalents) migrate to different poles.

FIGURE E.—Metaphase I showing trivalent composed of telocentric and two chromosomes 5. The orientation of the telocentric chromosome, indicated by arrow, is such that it will disjoin with a normal chromosome 5.

FIGURE F.—Late prophase in microspore hyperploid for the telocentric chromosome which is indicated by arrow.

FIGURE G.—Metaphase I showing 10 bivalents and unpaired telocentric chromosome which has congressed on the spindle.

FIGURE H.—Metaphase II with 10 dyads on the equatorial plate and a daughter univalent, arising from the equational separation of the telocentric chromosome in anaphase I, lying off the plate.

FIGURE I.—Anaphase II with 10 monads passing to each pole while a daughter univalent lags. Origin of daughter univalent same as in figure H.

FIGURE J.—Anaphase II with 11 monads passing to each pole. In the preceding anaphase the telocentric chromosome was a member of a trivalent group and underwent a reductional division. The two daughter univalents of the telocentric chromosome are rod-shaped because of their terminal centromeres while the other monads are V- or J-shaped.

EXPLANATION OF PLATE 3

FIGURE A.—Metaphase I showing that the centromere is divided at its poleward tip. Each chromatid will form its own half-spindle component but the bulk of the centromere does not appear to be divided.

FIGURE B.—Anaphase I showing centromeres divided at poleward tips. As in figure A the bulk of the centromere does not appear divided.

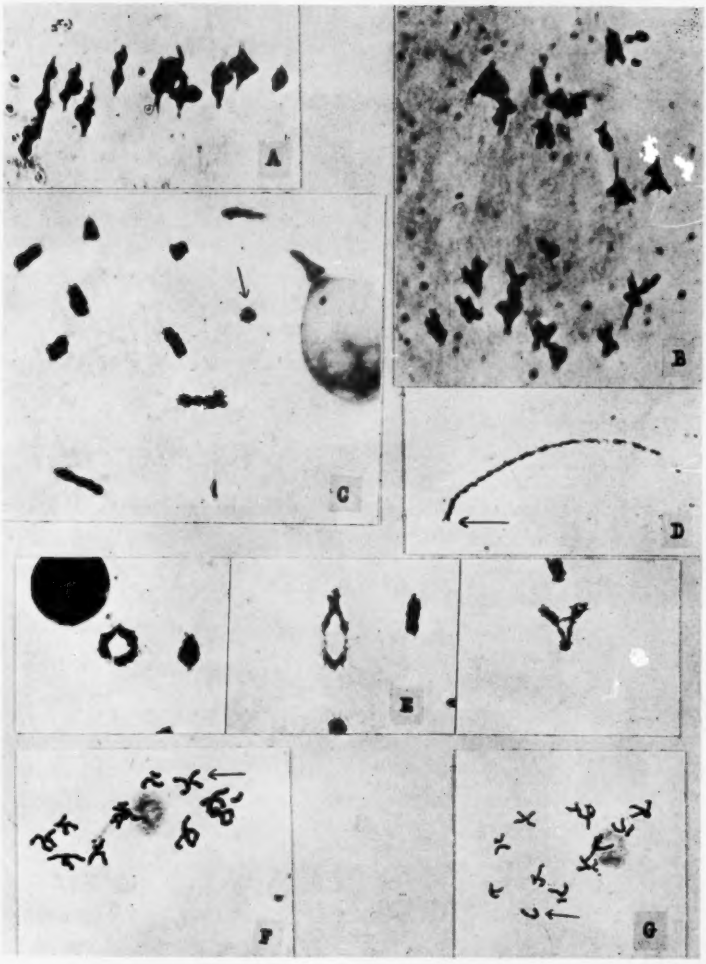
FIGURE C.—Diakinesis in secondary trisome with the secondary chromosome present as a ring of 1. This configuration results from chiasma formation between its two homologous arms.

FIGURE D.—Pachytene stage showing pairing of the two identical arms of secondary chromosome. The median centromere (see arrow) has a terminal position because of the synapsis of the two homologous arms. This configuration will give a ring of 1 at diakinesis.

FIGURE E.—Diakinesis in secondary trisome. Three ring configurations produced by pairing of secondary chromosome with the two normal chromosomes 5. These rings of 3 are produced by pachytene associations shown in Plate 1. In the leftmost ring terminalization is complete while it is only partially so in the middle figure and in the rightmost figure there has been little if any movement of the chiasmata.

FIGURE F.—Prophase II with the secondary chromosome indicated by arrow. Since the two arms of this chromosome are alike the dyad appears as an X-shaped element with all four arms of the X of equal length. In the dyad to the left of the secondary the two short arms appear continuous as do the two long arms. The unstained centromere lies between the two chromatids at the center of the X. The rod-shaped body to the right is a B-type daughter univalent.

FIGURE G.—Prophase II showing the two chromatids of a telocentric chromosome. Since the two chromatids appear continuous the centromere must be forced to one side at this stage. If the centromere was not strictly terminal an X-shaped figure would be produced at prophase II as is the case for the rest of the chromosome complement.


A
B
C
D
E
F
G

THE TEMPERATURE RESPONSES OF FLIES WITH THE DEFICIENCY VESTIGIAL-DEPILATE IN DROSOPHILA MELANOGASTER[1]

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Received April 23, 1940

INTRODUCTION

THE effects of temperature on a number of the genotypes involving the vestigial locus of *D. melanogaster* have been reported in earlier papers (HARNLY 1930a, b, 1932, 1933, 1936; HARNLY and HARNLY 1935, 1936). During these investigations it became evident that the critical temperatures for a marked increase in wing size vary with the genotype (there may be two critical points for one genotype) and that the phenotype of an individual is dependent on both its genotype and the temperature experienced by it during a critical period of development. Consequently, a given phenotype may be produced at different temperatures by a number of genotypes. For example, the "strap" wing phenotype was produced by the strap allele of vestigial at 25°C but may be produced by vestigial at 31° and 32°, by dimorphous vestigial at 25° and 29°, by vestigial-pennant/vestigial at 26° and 28°, by vestigial-Depilate/vestigial-pennant at 16°, etc. An hypothesis has been proposed for a definite pattern of wing form in replacement and development. This hypothesis is based on the data covering known gene substitutions at one temperature, a single genotype at different temperatures, and transfers between two temperatures for each individual during its development. The duration of the temperature-effective-period of wing development, the rate of the growth processes involving the wings during this period, and the general growth rate of the individual vary independently of each other through the viable temperature range. It has been possible to distinguish between the effect of the gene on growth and on differentiation through the independent changes in size, and the form of the wings at different temperatures. It has not been possible to distinguish clearly between the powers or effects of the two alleles in the heterozygote since the action of each alone was known only in the double dose of the homozygote. The work reported here and in a paper following is an attempt to determine something of the powers of alleles at the vestigial locus in the haplo-condition and so determine their respective rôles in the heterozygote. The experiments were performed several years ago immediately after the work on vestigial, dimorphos vestigial, pennant, and

[1] The author wishes to express his thanks to MISS RUTH RONES for technical assistance in this work.

pennant/vestigial while the stocks were isogenic from repeated intercrosses.

DESCRIPTION OF DEPILATE

Depilate (vg^D), formerly called $vg^{De\ 31\ a\ 22}$ by BRIDGES, removes a zone of microchaetae from the thorax, and hairs and bristles from the legs. It has been found to be a deficiency for vestigial (BRIDGES), for 1C (CURRY), and for scabrous (IVES). BRIDGES has found it shows a salivary chromosome deficiency for 49 C 1–49 E 2 inclusive. Classification and viability are excellent but it is lethal when homozygous. There may be a slight reduction of crossing over (D.I.S. number 4, Sept. 1935).

STOCKS AND METHODS

Vestigial-Depilate/Upturned flies were obtained from Dr. DEMEREC. These were crossed with a wild strain that had been repeatedly backcrossed to the inbred vestigial stock used in the temperature experiments mentioned above. In the F_1 and succeeding generations the $vg^D/+$ females were backcrossed to males of this special wild type. This produced a Depilate stock identical in its gene complex with the wild type, vestigial, dimorphos vestigial, and vestigial-pennant stocks which had been interbred and used in my work with temperature, differing from them only by the short deficiency in the region of the vestigial locus. Consequently, all my previous work with the vestigial locus should be comparable with the results obtained from mating these stocks to this specially bred Depilate line.

In the first series of tests, $vg^D/+$ females of this prepared line were mated to males of the special wild-type stock. Eight pairs per vial were allowed a $1\frac{1}{2}$ hour egg-laying period at 25°C. They were then repeatedly transferred to new vials for similar periods. The number of offspring per vial was below the point at which crowding influences the wing size and form. All the vials remained at 25° for 24 hours. At that time practically all viable eggs had hatched (HARNLY 1929). The vials were then distributed so that equal numbers of the offspring of each set of parents were placed in incubators set at 16°, 20°, 24°, 28°, 30°, and 32°C. All further development took place at the temperature specified with the exception of those individuals placed at 32°. This temperature is lethal for total development and the vials placed at 32° were returned to 25° after 120 hours (five days) of development for the completion of pupation and emergence of the adults. There were two types of offspring in these trials, $vg^D/+$ and $+/+$. They could be separated readily by the absence of microchaetae on the thorax of the $vg^D/+$ flies. In the second series of trials $vg^D/+$ females were mated to males of the inbred vestigial stock.

The handling of these trials was identical with that of the first series described above. The offspring were of two types: vg^D/vg $+/vg$ and could be separated by both the absence of microchaetae on the thorax of the vg^D/vg flies and the difference in wing size.

The air temperature variation within the incubators was $\pm 0.05°$ and the incubators were kept in a cold room (10°) which varied less than 1° in temperature. The accuracy of the incubators, the temperature control mechanism, the food and vials used, and the method of measuring the wings have all been discussed elsewhere (HARNLY 1936). Measurements were made of the right wing of 60 males and 60 females at each temperature for the genotypes $vg^D/+$ and vg^D/vg.

TEMPERATURE EFFECTS ON VESTIGIAL-DEPILATE/WILD

The mean wing lengths and areas of $vg^D/+$ flies reared at the temperatures indicated are recorded in tables 1 and 2. The wings of such flies vary

TABLE 1

Mean wing length in mm of vestigial-Depilate/wild flies, 3 trials.

°C	MALES		FEMALES	
	LENGTH ± P. E.	σ	LENGTH ± P. E.	σ
16	2.47±0.006	0.07	2.73±0.009	0.11
20	2.40±0.007	0.07	2.70±0.009	0.10
24	2.22±0.007	0.08	2.51±0.009	0.10
28	2.09±0.005	0.05	2.35±0.009	0.10
30	2.00±0.005	0.06	2.27±0.007	0.08
32	2.04±0.007	0.08	2.32±0.009	0.10

in size inversely with the temperature for both the males and the females. The wings of the females are regularly larger than those of the males. The decrease in wing size from 16° to 30° is the same for both sexes, the mean

TABLE 2

Mean wing area in sq. mm of vestigial-Depilate/wild flies, 3 trials.

°C.	MALES		FEMALES	
	AREA ± P. E.	σ	AREA ± P. E.	σ
16	1.64±0.009	0.11	1.91±0.012	0.14
20	1.55±0.007	0.09	1.95±0.010	0.12
24	1.34±0.007	0.08	1.67±0.011	0.12
28	1.17±0.005	0.06	1.45±0.010	0.12
30	1.08±0.005	0.06	1.34±0.008	0.09
32	1.12±0.007	0.09	1.41±0.010	0.12

length decreasing 0.5 mm and the area 0.6 sq. mm. No profound change in wing phenotype occurs and the post-scutellar bristles and balancers are normal throughout the temperature range examined. The change in slope of the curves for the males and the females between 30° and 32° is probably due either to the marked lengthening of the larval period at 32° (HARNLY 1936) or to a shift in dominance. A shift in dominance of pennant/vestigial has already been observed (HARNLY and HARNLY 1936). Between 20° and 30° the curves are practically straight lines. With the same parentage the adult yield from the vials at 30° ran some 50 percent behind that of the vials at lower temperatures. Equal proportions of +/+ and $vg^D/+$ would be expected from a backcross and were obtained at all the points except 30°. There the frequency of the $vg^D/+$ compared to their +/+ sibs was definitely below expectation. Apparently the deficiency had lowered the lethal point significantly.

TEMPERATURE EFFECTS ON VESTIGIAL-DEPILATE/VESTIGIAL

The mean values for wing length and area of vg^D/vg flies are given in tables 3 and 4. The curves for vg^D/vg give the appearance of very little change in wing size through the 16° range examined in this report. However, a consideration of the data in tables 3 and 4 shows very definite

TABLE 3

Mean wing length in mm of vestigial-Depilate/vestigial flies, 3 trials.

°C	MALES		FEMALES	
	LENGTH ± P. E.	σ	LENGTH ± P. E.	σ
16	0.57±0.011	0.11	0.57±0.008	0.09
20	0.58±0.009	0.10	0.58±0.007	0.07
24	0.57±0.008	0.09	0.65±0.007	0.08
28	0.62±0.008	0.09	0.70±0.006	0.07
30	0.68		0.78	
32	0.71±0.004	0.05	0.76±0.006	0.07

changes in wing size for both sexes. The wings of the males lengthened 25 percent over their lowest mean value and enlarged 56 percent in area; the increases over the lowest mean value for the females are 37 percent in length and 89 percent in area. These total changes are not due to a uniform increase between 16° and 32°. The wings of the males did not lengthen between 16° and 24° but did lengthen regularly between 24° and 32°; the curve being practically linear through that temperature range. The area of the wings increased more or less regularly from 16° to 32°. The wings of the females lengthened only slightly between 16° and 20° but quite definitely above that point, the curve between 20° and 32° being practically

TABLE 4

Mean wing area in sq. mm of vestigial-Depilate/vestigial flies, 3 trials.

°C	MALES		FEMALES	
	AREA ± P. E.	σ	AREA ± P. E.	σ
16	0.09±0.003	0.03	0.09±0.002	0.02
20	0.11±0.003	0.03	0.11±0.002	0.02
24	0.11±0.002	0.03	0.14±0.003	0.03
28	0.12±0.002	0.03	0.16±0.002	0.03
30	0.14		0.16	
32	0.14±0.002	0.02	0.17±0.002	0.02

a straight line. Their wings increase regularly in area from 16° to 32°, the amount being almost constant for each 4° rise in temperature. As in the case of the wings of *vg/vg*, the wings of vg^D/vg (haplo-vestigial) flies vary directly in size but not always proportionately with the temperature. The wings of the females are larger in length and area than those of the males at 24° and all temperatures above that point, a sexual dimorphism found in +/+, +/*vg*, $vg^D/+$ and vg^p/vg^p but not found consistently between 24° and 32° for *vg/vg*. The wings of the vg^D/vg females attain a length at 24° typical for vestigial and the males do likewise between 28° and 30°, but even at 32° neither sex develops wings typically vestigial in area. The balancers (halteres) remain mere vestiges and the post-scutellar bristles point cephalad between 16° and 32° showing none of the changes that occur in these structures of *vg/vg* through this temperature range.

The yield of flies and the proportion of +/*vg* to vg^D/vg were normal for a backcross between 16° and 24° but at 28° the vg^D/vg class was approximately 25 percent of expectation. At 30° only 19 vg^D/vg flies emerged from some 90 vials. The frequency of survival at 28° for vg^D/vg is approximately that of *vg/vg* at 31°. Evidently the lethal point was lowered approximately 3° by this deficiency in the vestigial animals. One cannot depend on the wing values at this temperature because of the very few individuals obtained at 30°. However, after 120 hours of development at 32° followed by transfers to 25° for the completion of development the flies emerged in the usual equal proportions of the two phenotypes from a monohybrid backcross, and the expected yields were obtained. The wing values at 32° are based on satisfactory yield, ratio of types, and number of individuals and can be considered significant. The profound increase in wing size characteristic for *vg/vg* at this point did not take place in the vg^D/vg individuals.

TEMPERATURE AND WING FORM

The wings of the original vestigial-Depilate/Upturned stock are wild-

type in size but show nicks regularly in the distal margin between the second and third, and third and fourth longitudinal veins. Approximately 20 percent of them have one wing with a complete margin and some 7 percent have perfect margins on both wings. If lateral nicks occur at 24° they must be extremely rare as none were noted in over six hundred individuals examined.

In the work reported here wings similar to those just described were developed at all temperatures by the prepared $vg^D/+$ stock and these wings were also phenotypically identical with those previously reported and figured for vg^p/vg^p (figure 3, HARNLY and HARNLY 1936). Again in the case of $vg^D/+$ the phenotype does not vary markedly through the temperature range examined. The only detectable change was in the frequency of nicks on the lateral margins and of wings with perfect margins. The frequency of right wings with one or more nicks in the lateral margins dropped rapidly: males 16°—38 percent, 20°—17 percent, 24°—8 percent, and 28°—3 percent; females 16°—65 percent, 20°—43 percent, 24°—17 percent, and 28°—7 percent. Lateral nicks were not observed in the wings of the males at 30° and 32° and on only one female at 30° with none showing at 32°. Wings with lateral margin nicks and no distal nicks were found only at 16° and occurred five times in 120 observations. The frequency of either a right or a left wing or both wings with perfect margins increased from 15 percent at 16° to 33 percent at 28° for the males, and from 10 percent at 16° to 65 percent at 30° for the females. The disappearance in these flies of lateral margin nicks followed by a marked increase in freedom from distal nicks is further evidence for our hypothesis of pattern in wing development and replacement by gene substitutions. This hypothesis is based on our data from partial development of vestigial at a high temperature with completion at a lower temperature (HARNLY 1936), the phenotypic changes of a single genotype through the viable temperature range (HARNLY and HARNLY 1935, 1936), and the results of MOHR (1932) from allelic substitutions at a single temperature. This hypothesis assumes growth or replacement first distally through the region of the second, third and fourth longitudinal veins, followed by a lateral completion of the wing, the distal margin in the region of the third and fourth longitudinal veins being the last to develop or to be replaced (HARNLY and HARNLY 1935, HARNLY 1936). It must be noted in connection with this hypothesis and the data presented above for $vg^D/+$ that vestigial-pennant developed perfect margins on one or both wings only at 16° and above this point the frequency of wings with defective lateral margins mounted rapidly.

The data from those genotypes producing at least occasional perfect wings make possible the consideration of an interesting question. Are the wing buds of the left and right mesothoracic discs each self-determining,

TABLE 5

Frequency with which both wings are normal (per 1000) in vestigial-Depilate/wild flies.

°C	MALES		FEMALES	
	CHANCE	OBSERVED	CHANCE	OBSERVED
16	7	17	6	50
28	33	33	133	170
30	6	18	228	308
32	3	33	66	133

or are they dependent, at least in part, on materials or events originating outside of the discs? One might assume that if they are self determining the frequency of two perfect wings on the same fly would be that of chance calculated in terms of either of two events happening separately (left, right), or both events happening simultaneously. If they are not self-determining the frequency of both wings perfect might be some other value than that expected by chance. Each occurrence of left normal, right normal, and both wings normal was recorded for all of the $vg^D/+$ flies examined at each temperature in this work. From these data were calculated the frequency by chance and the observed frequency of both wings normal (table 5). Similar calculations on a test of the original vestigial-Depilate/Upturned stock at 24° produced for the males, values of 30 by chance and 73 observed; and for the females, values of 29 by chance and 69 observed. Our earlier data on pennant/pennant at 16° (HARNLY and HARNLY 1936) when calculated gives: males chance 101, observed 180; females chance 100 and observed 171. It is obvious that for at least these three genotypes the frequency of both wings perfect is far greater than is expected by chance. Consequently it is evident that the wing buds are not self determining.

Since they are not self determining there must be some outside factor involved. Some other organ may produce a material essential for wing bud formation which reaches the discs by way of the body fluids. A rather extensive list of organs have been tested by micro-injection with negative results. These tests are being continued.

The difference between the expected by chance and the observed frequency of both wings perfect varies with both the genotype and the temperature. The observed frequency was eight times the frequency by chance at 16° for $vg^D/+$ females but only 1.7 times expectation for vestigial-pennant females at the same temperature. The variation with temperature for a single genotype has been shown above. An investigation along these lines of eight additional genotypes is nearing completion and the results with their implications will be reported in the near future.

The vg^D/vg wings of both sexes at 30° and 32° are vestigial in phenotype, but slightly narrower than those of homozygous vestigial flies reared between 24° and 28°. The progressive decrease in the size of the wings of the vg^D/vg flies with a fall in temperature results in a definite change in phenotype between 32° and 16°. What little is left of the basal region of the wild-type wing in the phenotype known as vestigial, is reduced at lower temperatures in vg^D/vg flies and practically disappears at 20° and 16°. All that remains in extreme cases is the shoulder jutting out from the thorax bearing the vein formed by the fused bases of the I, II, and III longitudinal veins. This vein is surrounded by a thin envelope of wing tissue. A wild-

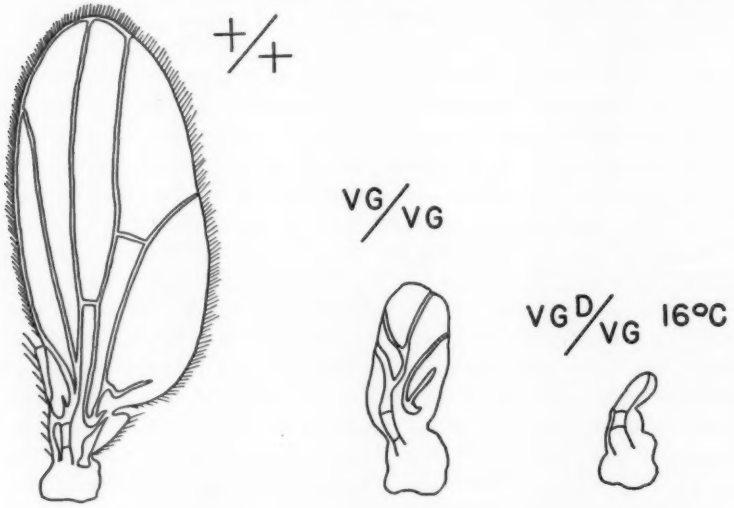

FIGURE 1.—Wings of wild type, vestigial and vg^D/vg flies at same magnification; 16°C.

type wing, a typical vestigial wing, and this rudiment found on vg^D/vg at low temperatures are all drawn at the same magnification in figure 1.

DISCUSSION

STANLEY (1935) has reported a detailed examination of +/+ and +/*vg* wing length through the viable temperature range. A comparison of his values for these two genotypes with the data reported here for $vg^D/+$ shows a close agreement for the three genotypes in degree of sexual dimorphism, trend of the curves, values for wing length at each point, and total change in wing length. The modifiers present in his stocks must differ from those in mine and are probably the cause of the slight differences in

our values at some points. Obviously "dominance" in these cases means that one wild-type gene at the vestigial locus (in collaboration with the other normal wing genes) follows the wild type response to temperature and is capable of producing wild-type wings. This is true if the other second chromosome carries either a recessive allele at the vestigial locus or a short deficiency including it. However, $+/vg^D$ (Df [2] vg^D,—bands 49 C 1–49 E) is normal in phenotype while $+/vg^S$ (Df [2] vg^S,—bands 49 C 1 to just to the left of 50 A) has markedly abnormal margins at 25° (MOHR 1932). The normal phenotype and "dominance" depends on the extent of the deficiency and the exact loci absent.

Between 16° and 28° the curves for $vg^D/+$ males and females coincide with those of vestigial-pennant, a regressive mutation from vestigial to a recessive wild phenotype (HARNLY and HARNLY 1936). But at 30° the wings of the homozygous vestigial-pennant males and females are respectively some 0.2 mm and 0.3 mm shorter than those of the male and female $vg^D/+$ flies. In our report on vestigial-pennant it was suggested that there must be a critical temperature for change in wing size between 28° and 30° for this allele. There is no similar sharp inflection between 28° and 30° of the curves from the data presented here for $vg^D/+$ nor in the curves of STANLEY for $+/+$. If the apparent critical point between 28° and 30° for vestigial-pennant is real, then it constitutes an additional difference from the wild type in the mutation from vestigial to a recessive wild-type allele. We have reported previously a number of differences between this recessive wild-type reversion and the dominant wild-type allele (HARNLY and HARNLY 1936).

In the case of the $+/vg$ heterozygote the normal allele together with the other wild-type wing genes produces the normal wings and apparently prevents the inception of the wing-forming processes by the vestigial allele in the heterozygote. This would be the obvious interpretation of the $+/vg$ response when it is compared with that of wild and $vg^D/+$ through the temperature range examined, and one considers the temperature-effective-periods in figure 17 of STANLEY'S (1935) paper. In that figure the termination of the temperature-effective-period of vestigial and the inception of that period for wild barely overlap at 17° and 27°, but the effective-period for $+/vg$ coincides almost exactly with that of the wild homozygote at 17° and again at 27°. Several explanations may be offered for the failure of the vestigial gene in the heterozygote to initiate the processes involved in wing development. It is possible that the wild allele "inhibited" the earlier action of the vestigial gene. At present there is no evidence for such an assumption regarding the wild allele. From figure 1 and tables 3 and 4 in this paper it is obvious that a single vestigial gene produces very little wing material below 28°. The effect on wing size of the single vestigial gene

in the $+/vg$ larvae may have been so small that it was not detectable in the measurements of the wings of the heterozygotes transferred earlier than the beginning of the effective period for the wild-type allele. A third possible explanation is a shift in the time relationships of the activities of the vestigial gene when heterozygous with different alleles and associated with various modifiers. Marked shifts in the critical temperature under such conditions have already been demonstrated (HARNLY and HARNLY 1935, 1936). It may be that from one genotype to another similar changes occur in the time relationships during ontogeny. The detailed discussion and acceptance or rejection of a time relation hypothesis will have to await the appearance of further data on genes at this locus. Data on the temperature-effective-periods and on the development of the wing buds of the mesothoracic discs of several more genotypes will be available in the near future.

The size of the wings of the vg^D/vg and the vestigial flies varies directly with the temperature at which the flies develop. Critical temperatures for a marked increase in the length of the wings have been found for homozygous vestigial males at 30° and for the females at 31° (HARNLY 1930, STANLEY 1931). A comparison of the curves for wing length and area of vestigial with those of vg^D/vg shows one profound difference between the two genotypes in their response to temperature. The very sharp inflection in the curves of vestigial at a high temperature does not occur in the curves for vg^D/vg. There is no significant change in the slope of the wing area curves for these heterozygous males and females between 16° and 32°. The wings of the males do not increase in length between 16° and 24° but do lengthen regularly above that point. The wings of the females do not change between 16° and 20°, lengthening regularly through all higher temperatures. Any increase in length was apparently dependent upon the previous completion of the very basal region of the wing (figure 1). From the data above it is evident that there was no critical temperature for the increase in wing area of the heterozygous flies, the wings enlarging uniformly throughout the temperature range examined. Critical temperatures between 20° and 24° for the females and 24° and 28° for the males for increase in wing length may be assumed from the data and the curves. At present I would rather not call these points critical temperatures. They do not show the profound changes associated in vestigial with a rise of one degree nor do they appear to correspond to the marked lowering of the critical temperature by the introduction of the sex-linked dimorphos gene into the vestigial genotype which we have reported previously (HARNLY and HARNLY 1935). These points of inflection in the curves for length of the wings of vg^D/vg are not conditioned apparently in the same way as those of homozygous vestigial, vestigial-pennant, dimorphos vestigial, or

the heterozygote vestigial-pennant/vestigial. Critical temperatures, as I have used the term to date for these genotypes, implies a marked change in the phenotype at a one degree interval. In terms of the data of the temperature-effective-periods it is interpreted as a marked change in duration, rate, or both of certain developmental processes affecting specific organs or structures and is not accompanied by a proportionate change in the duration of the developmental period of the entire organism. It is the temperature at which a change occurs in the wing determining activities with no change in the general rate and duration of developmental activities of the genotype. In the case of vg^D/vg there is no change in slope of the area curves. The conditioning factor in the length curves for a change in slope seems to be merely the attainment of a certain breadth of wing or amount surrounding the basal vein. Temperature is a secondary factor acting indirectly on length through its direct effect on area where it shows no critical point. This difference between the response of vestigial and vg^D/vg at high temperatures calls for an explanation.

The critical temperature reported by both STANLEY and HARNLY for vestigial may not be due to the vestigial gene. Instead, the temperature response might be controlled by one or more other loci closely associated with the vestigial locus in the second chromosome. The apparent disappearance of the critical temperature for a marked increase in the size of the wings of the vg^D/vg flies would then be due to the absence of other genes in the vestigial-Depilate chromosome close to the deficiency at the vestigial locus. There are certain facts in disagreement with this assumption. The stocks used in the series of papers (1930–36), including the vestigial-Depilate stock, have all been interbred to produce genotypes identical except for the specific genes under examination. Since the crosses reported here were $vg^D/+$ to wild, and to vestigial, the Depilate chromosomes were identical in the two sets of data.

It was noted above that $vg^D/+$ produced curves super-imposable on those of STANLEY for wild through the temperature range examined. The absence of one set of genes in the immediate vicinity of the vestigial locus had caused no change in the temperature response. If the non-appearance of a critical temperature in the vg^D/vg data is attributed to the haplo-condition of genes in the vestigial chromosome then it must be assumed that these genes differ from those in the wild-type chromosome. But this cannot be true because the wild-type and vestigial chromosomes had been carried together in heterozygous females for many generations.

The assumption of genes determining the temperature response being closely linked to the vestigial gene is very weak historically and there are no data to support it. As early as 1918 ROBERTS reported that high temperatures had a profound effect on the expression of homozygous vestigial.

Subsequently sub-lethal or semi-lethal temperatures were used with vestigial in a number of laboratories in routine class experiments to demonstrate the effect of the environment on the expression of the gene. Later on identical critical temperatures were reported in genealogically widely divergent stocks of vestigial flies (HARNLY 1930, STANLEY 1931). If the critical point is determined by a gene linked to vestigial then in twenty years one would have expected that in some stock a crossover or mutation would have occurred resulting either in a stock showing no response to temperature or else one showing a marked effect at some other temperature. In all the stocks of vestigial used such a change has never occurred. Furthermore, PEARL (1928) in his experiments on longevity was unable by repeated outcrosses to dissociate any of the morphological or physiological effects attributed to the vestigial gene. The historical facts support only one assumption—namely, that in the case of vestigial the direct but disproportionate variation in wing size with temperature is a characteristic of the vestigial gene. It has already been demonstrated that the critical point is capable of being either markedly or slightly shifted by different modifying genes (HARNLY and HARNLY 1935, STANLEY 1935). But the breeding methods used and the facts discussed above prevent offering modifying genes as an explanation of the absence of a critical point for wing size at a high temperature in the vestigial-Depilate/vestigial data. A more probable explanation may be found in the facts already known about the vestigial gene and in its relationship to the genotype as a whole.

The presentation of general considerations on gene action during development as determined from temperature studies will appear in the forthcoming paper on vestigial-Depilate/vestigial-pennant. At that time the data on the temperature responses and temperature-effective-periods of several more genotypes will be available to the reader from other papers to appear shortly.

SUMMARY

1. The wing length and wing area of male and female vestigial-Depilate/wild flies varies inversely with the temperature. The wings are practically normal, and the balancers and the post-scutellar bristles are normal throughout the temperature range examined. The mean wing lengths and areas are practically identical with those reported by STANLEY for wild type and wild/vestigial and by us for vestigial-pennant. The deficiency had lowered the lethal temperature.

2. The wing length and wing area of vestigial-Depilate/vestigial flies vary directly with the temperature for both sexes. The wings vary from minute stubs to vestigial in phenotype. The halters and the post-scutellar bristles are not normal from 16° to 32°. The marked increase in wing size

found for vestigial at high temperatures did not occur in vestigial-Depilate /vestigial. The lethal temperature was lowered some 3°.

3. Data presented indicates that the left and right dorsal mesothoracic discs are not self-determining.

4. A small deficiency in the region of the vestigial locus paired with a normal wild-type second chromosome can produce a wild-type wing. A wild genotype heterozygous for a slightly larger deficiency in the same region produces a markedly abnormal wing. The normal phenotype and dominance depend on both the extent of the deficiency and the exact loci absent.

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THE STRUCTURE OF SALIVARY GLAND CHROMOSOMES OF DROSOPHILA MELANOGASTER IN EXCHANGES BETWEEN EUCHROMATIN AND HETEROCHROMATIN

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INTRODUCTION

IN RECENT years SCHULTZ (1938) and PROKOFYEVA-BELGOVSKAYA (1937) have published the results of studies on certain rearrangements in the salivary chromosomes of *Drosophila melanogaster*, in which exchanges of material have occurred between euchromatic and heterochromatic parts of the chromosomes. These authors have both concluded that such rearrangements tend to affect the structure of the bands adjacent to the breaks.

PROKOFYEVA-BELGOVSKAYA (1937) states that "a transfer of any chromosome section to the chromocentral region modifies the structure of that section into a chromocentral structure. . . . Conversely, the removal of chromosome sections of the inert region from the chromocenter (by an inclusion into the active region) brings about a change in the cytological structure of the translocated sections; they become indistinguishable from the active part of the chromosome."

SCHULTZ's results from direct observation and ultra-violet photography do not show a constant modification of bands, but he claims that euchromatic bands transferred to the chromocenter are frequently intensified, showing an increased nucleic acid content. Less frequently the bands nearest to the heterochromatic region are not visible, owing either to their complete loss or to their structure having become indistinguishable from that of the chromocenter; while bands a little further away are more intense than they appear to be in the normal chromosome.

At the suggestion of DR. DEMEREC, a further study of the problem has been made from material at hand in this laboratory. The results are presented here.

MATERIAL

The following rearrangements were studied:

A. Insertions of 3C bands into chromocenter.

1) Dp(1; 3) 264–58; a duplication of a small euchromatic section of X (3B 3·4 to 3D 5·6 inclusive) which is inserted in the chromocenter of 3L proximal to 80C (Plate 1, A).

* The cost of the accompanying plates has been borne by the Galton and Mendel Memorial Fund.

2) T(1; 2; 4) 264–85, a complex translocation in which a euchromatic section of X (3C1 to 6A 1·2 inclusive) is inserted in the chromocenter of 4 between 101F and 102A. (Plate 1, B).

3) T(1; 4) 264–86, a translocation in which a piece of X (3C7 to 3E5 inclusive) is inserted in the chromocenter of 4 at 101F (Plate 1, C).

4) T(1; 3) 264–100, insertion of a piece of X (3C1 to 4B 3·4 inclusive) in the chromocenter of 3 between 80C and 81F (unmapped region). (Plate 1, E).

B. Translocations of 3C bands to heterochromatic regions.

5) T(1; 3) 264–70, a translocation in which heterochromatin of 3L is adjacent to 3C4 and 3C 5·6 (Plate 1, D; Plate 2, G).

6) T(1; 2; 3) 264–74, a complex translocation in which 3C 9·10 is adjacent to heterochromatin of 2L. (Plate 1, F).

C. Insertions of 3C bands into euchromatic regions.

7) Tp(1) 264–63, a transposition in which 3C 9·10 and the succeeding bands are adjacent to 13B.

8) T(1; 2; 3) 264–87, a complex translocation with a piece of X (3C 9·10 to 10A 1·2 inclusive) inserted in 2R between euchromatic segments 60A and 45E.

9) T(1; 3) 258–44, a complex translocation with a piece of X (3C4 to 4D 1·2 inclusive) inserted in 2R between 56 E1 and F1.

D. Insertions of other regions into chromocenter.

10) T(1; 3) 268–37, insertion of a piece of X (5D 3·4 to 7B 1·2 inclusive) in 3L chromocenter proximal to 80C. (Plate 2, H).

11) T(1; 2; 3) 268–40 a complex translocation with a piece of 3R (87D3 to 88C 2 inclusive) inserted in the chromocenter of 2L between 40F and 41A (Plate 2, J).

E. Insertion of heterochromatin into euchromatic region.

12) T(1; 2) 258–36, a complex translocation in which a heterochromatic section of 2R (41B to 41F) is inserted between two euchromatic regions of X, following 3C 5·6 and preceding 4C 3·4 (Plate 2, I).

F. Rearrangement involving 2B region and chromocenter.

13) In (1) 313–25–36. Inversion of a section of X from 2B ("bulb" region) to the chromocenter. (Plate 2, K).

METHODS

Slides of the salivary gland chromosomes of female larvae heterozygous for the rearrangements concerned were prepared by both aceto-carmine and Feulgen techniques. The larvae were raised in the cold room at a constant temperature of 18°–19°C.

For analysis of the slides, the equipment consisted of a 90×, 1.3 N. A. apochromatic objective, an oil-immersed 1.4 N. A. condenser, 12.5×

compensating oculars, and a Bausch & Lomb research lamp with the green Wratton filter number 61 which transmits light waves between 4500 and 6100 A° and has the maximum at about 5200 A°.

Drawings were made with the aid of a camera lucida at an initial magnification of c. 3000×.

Cytological analyses were made with the aid of BRIDGES' (1935; 1938) salivary chromosome maps.

OBSERVATIONS AND CONCLUSIONS

It must be admitted that observations on changes such as these are difficult, because the chromocenter region is very easily broken up by the pressure used in making slides, and the loosely organized bands may be smeared over adjacent chromosome sections so as to obscure the euchromatic bands. Figure F on Plate 1 shows an unquestionable example of this. I have interpreted several figures in this way, though they might conceivably be interpreted as being deficient in one or more euchromatic bands immediately adjacent to the chromocenter.

In several of the rearrangements (numbers 1–6 described above) the euchromatic bands involved were those of the white-Notch region (section 3C of the X chromosome). These bands are very well defined and easily recognizable (Figure 1 second from top). In each case a break had been induced in this region, and one or both of the broken ends had become attached to a heterochromatic region where a second break had occurred. The change was associated with mottling of one or more characters determined by genes in the white-Notch region.

At the temperature at which the larvae were raised (18° to 19°C), mottling was extreme (most of the tissue showing the mutant character, with a few patches of wild-type tissue). The slides were examined carefully for the presence of extra Y chromosomes which, according to SCHULTZ, increase the amount of wild-type tissue in phenotypic characters and reduce the variation in the appearance of the salivary bands. No extra Y chromosomes were found in the salivary glands used in this study.

In Dp(1) 264–58, where twenty euchromatic bands of X were inserted in the 3L chromocenter, figures were seen in which all the bands were readily identifiable and the appearance of these bands in their new position was strictly comparable with that in their normal position (Plate 1, A). The same was true in T(1; 2; 4) 264–85, where forty bands of X, beginning

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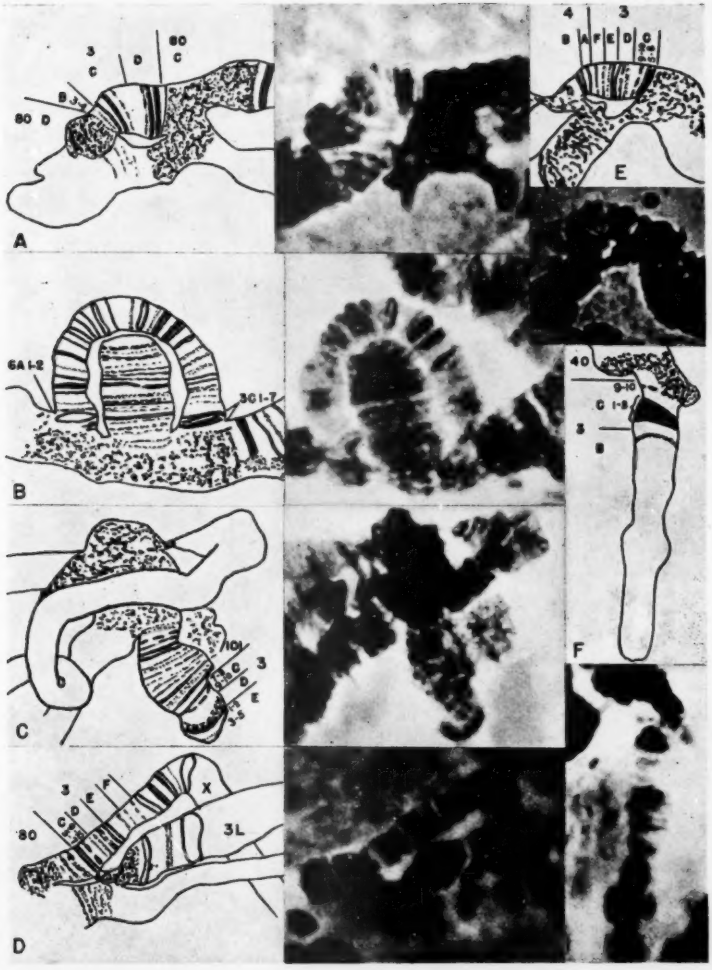
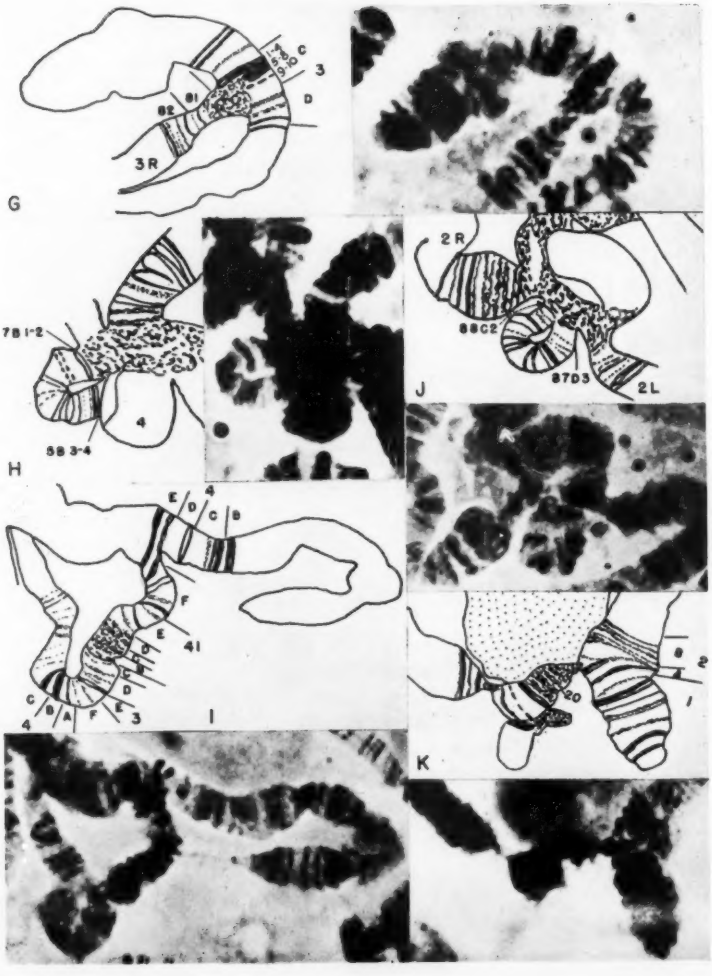
EXPLANATION OF PLATE 1

Photomicrographs and diagrams of Drosophila salivary chromosomes with following chromatin exchanges, A. Dp(1;3) 264.58; B. T(1;2;4) 264.85; C. T(1;4) 264.86; D. T(1;3) 264–70; E. T(1;3) 264.100; F. T(1;2;3) 264.74. For further description, see text.


A
B
C
D
E
F
6A 1-2
3C 1-7
10
3L
X
40
9-10
C 1-2


3R
82
81
C
B
3
D
G
2R
88C2
87D3
2L
J
7B1-2
4
88 3-4
H
E
D
C
B
4
F
E
41
C
B
A
F
E
3
4
I
20
2
1
K

at the 3C region, were inserted in the chromocenter of the fourth chromosome (Plate, 1, B); and in T(1; 4) 264–86 and T(1; 3) 264–100, which showed similar insertions of different lengths (Plate 1, C and E).

Occasionally a band near to a heterochromatic region appears darker than the corresponding band in the normal chromosome; but in other cells the same band may be lighter than normal. In T(1; 3) 264–70 part of the 3L chromocenter was attached to X before 3C 5·6 (Plate 1, D) and though the 3C bands were unaffected, the next group of dark bands in X (3D–E) following the heterochromatic (80D) region of 3L appeared darker than normal in some cells. A count was made to determine the proportion of such occurrences, with the following results:—

3D–E similar to normal—33 figures
3D–E darker than normal—5 figures
3D–E lighter than normal—1 figure

It seems probable that the variation in intensity, occurring in both directions, is due to random variation in the distribution of stain rather than to a specific effect of the neighboring heterochromatin.

As a control, a study was made of Tp(1) 264–63, T(1; 2; 3) 264–87, and T(1; 3) 258–44, in all of which sections of X including the 3C bands were inserted between euchromatic bands. In some figures homologous bands in the normal and reconstituted chromosomes showed with a different intensity.

In two of the translocations described above (Dp (1; 3) 264–58 and T(1; 4) 264–86) the limiting euchromatic bands at one end of the piece inserted in heterochromatin were light bands (Plate 1, A and C), while in all other cases the euchromatic band nearest to the heterochromatin was a heavy band. It was found that the light band 3B 3–4 in Dp(1; 3) 264–58 and the light bands 3F 3–5 in T(1; 4) 264–86 were not always visible; but this cannot be considered remarkable, as even in their normal position these bands are distinctly seen only in the best preparations. Wherever the euchromatic band nearest to the heterochromatin was heavy, this band was nearly always visible, and was indistinguishable only when obscured by spreading of the chromocenter.

Two other rearrangements showed insertions of other regions than 3C into the chromocenter. In one of these (T(1; 3) 268–37) an insertion of a piece of X was limited on both sides by heavy bands which could always be clearly seen (Plate 2, H). In the other (T(1; 2; 3) 268–40) a piece of 3R

EXPLANATION OF PLATE 2

Photomicrographs and diagrams of Drosophila salivary chromosomes with following chromatin exchanges, G. T(1;3) 264.70; H. T(1;3) 268.37; I. T(1;2) 258.36; J. T(1;2;3) 268.40; K. In(1) 313.25.36. For further description see text.

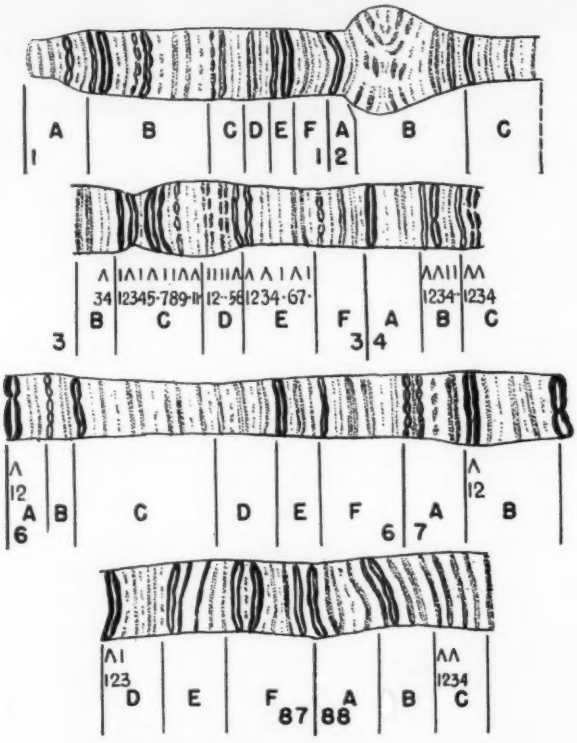


FIGURE 1.—Diagrams from Bridges' salivary chromosome maps showing three regions of X and (bottom) one of 3R, involved in translocations to heterochromatin; for comparison with drawings and photographs in Plates 1 and 2.

(figure 1 bottom) was inserted in the 2L chromocenter, and was limited at one end by the light bands 87D 3–8, at the other by 88C 2, a band of medium intensity. The six light bands in 87D cannot always be distinguished individually, but all figures showed that some, if not all, of the group were present (Plate 2, J), while 88C 2 was clearly visible.

These rearrangements provided no evidence for an effect of heterochromatin on the neighboring euchromatic bands, apart from the disturbed figures whose interpretation has been discussed above.

The converse effect of euchromatic regions on heterochromatic insertions was not apparent in this material. In T(1; 2) 258–36 part of the 2R chromocenter was inserted in X between the 3C 5–6 and 4C 1–2 bands, and the structure of the heterochromatic bands remained the same as it is when they are in the normal position (Plate 2, I). T(1; 3) 264–70 and T(1; 2; 3)

264–74, in which heterochromatic regions of other chromosomes were translocated to the 3C region, showed no change in the heterochromatin (Plate 2, G).

Evidence of change was found, however, in one rearrangement provided by DR. B. P. KAUFMANN. This was an inversion in the X chromosome in which the left break was in the 2B ("bulb") region (see figure 1, top) and the right break was in the chromocenter. The 2B bands were reattached in an inverted position to the chromocenter, and they frequently showed darker, but never lighter, than in the normal strand. Only one slide was available as it came from an experiment in which F_1 larvae from irradiated males were used for slides; in eighteen clear figures the bands in both strands of X appeared to be of like intensity, while in ten figures the 2B bands adjacent to heterochromatin were darker than those in the normal position. (Plate 2, K). No loss of bands was apparent in any of the figures.

According to PROKOFYEVA-BELGOVSKAYA (1938), the "bulb" region of X is peculiar in that it consists of inert material homologous with the chromocenter; and if this is so, the exceptional case just described cannot be considered as an example of the influence of heterochromatin on euchromatic bands.

It does not seem necessary to assume that the darkening of the bands is caused by the proximity of heterochromatin. If it were, the 3C region, which also is supposed by PROKOFYEVA-BELGOVSKAYA (1939) to consist of heterochromatic material, should be changed when transferred to the chromocenter; but as the bands of this region remain unchanged, the effectiveness of heterochromatin in producing such changes is open to question.

Whether the 3C region be considered as heterochromatic and comparable to 2B or as a series of euchromatic bands with which known loci are associated, the constancy of these bands tends to refute the suggestion of a heterochromatin effect. The evidence presented here as to the behavior of other euchromatic regions casts further doubt on the existence of such an effect.

As the observational methods used for this work are liable to subjective errors, it is possible that the photometric methods of CASPERSSON and SCHULTZ (1939) would give different results; and a further study of the material is being made by the latter methods.

SUMMARY

In a study of euchromatic bands transferred to heterochromatic regions, and vice versa, evidence of change in structure was found in a single case, where bands from 2B transposed to the chromocenter of X sometimes appear darker than the homologous bands in the normal X. Bands from the

white-Notch region of X (3C) do not appear to be lost or changed when they are adjacent to heterochromatin, nor do bands from some other euchromatic regions of the chromosomes.

Heterochromatic regions inserted between euchromatic bands retain their characteristic structure.

It is concluded that these studies provide no evidence of visible change or loss of bands due to an interaction between euchromatin and heterochromatin.

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